DEVELOPMENT OF A TRITIUM EXCHANGE
METHOD FOR DETECTION OF
DRUGS OF ABUSE

Final Report

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January 1, 1973 - July 31, 1974

MASTER

PREPARED FOR THE U. S. ATOMIC ENERGY COMMISSION
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PREFACE

The study described in this report has been under the direction of Dr. James J. Downs, Principal Chemist, Midwest Research Institute. The bulk of the study was performed at Panametrics, Inc., 221 Crescent Street, Waltham, Massachusetts 02154, on Subcontract No. 3751-1 under AEC Prime Contract No. AT(11-1)2292. The report was prepared by Dr. Philip Goodman, Dr. Abraham Rennert and Dr. Downs.

Approved for:

MIDWEST RESEARCH INSTITUTE

H. M. Hubbard, Director
Physical Sciences Division

31 July 1974

ABSTRACT

An investigation was undertaken to test the feasibility of using a combination of high pressure liquid chromatography (HPLC) and tritium exchange (TX) techniques for the quantitative analysis of common drugs of abuse and their metabolites. The prototype drugs used were amphetamine · HCl, methadone · HCl, morphine and secobarbital. The HPLC instrument was a standard commercially available single column type. The HPLC effluent was passed through the TX system which consisted of a teflon lined stainless steel column packed with the TX polymer followed by the ionization chamber radiation detector. Carrier liquids possessing active hydrogen atoms in their molecular structure would be tritiated by the TX polymer and therefore could not be used. The choice of HPLC carrier and column was further restricted by the necessity to use a polar system and not use coated columns. Working within these constraints a chromatographic system which was capable of properly separating the prototype drugs could not be obtained.

It was demonstrated that secobarbital would be tritiated by the TX polymer but detection of drugs using the HPLC-TX system could not be achieved. Some problems encountered were, (1) presence of water in the carrier fluid, (2) presence of impurities containing active hydrogens in the carrier fluid, and (3) build-up of contamination in the ionization chamber. Efforts to overcome these problems or to reduce them to allowable levels were only partially successful.

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SECTION I

INTRODUCTION

Drug usage, legal and illegal, on a national level has brought with it a variety of problems of a sociological and scientific nature. The scientific problems include, amongst others, determination of the short and long range physiological effects, studies of metabolic pathways and products, and studies of drug longevity in the human system. In addition, there are the problems of diagnosing drug abuse by noncomatose and comatose patients or drug use by patients in drug rehabilitation programs such as methadone maintenance. All such endeavors rely heavily upon the ability of the clinical scientist to separate, detect, identify and quantitate these drugs and their metabolites in serum or excretions.

Many methods, instrumental and noninstrumental, have been evaluated and some are presently being routinely used to perform the qualitative and quantitative analysis of drugs and their metabolites. These include gas chromatography (GC), thin layer chromatography (TLC), mass spectrometry (MS), coupled GC-MS, fluorescence spectroscopy and radioimmunochemical assay. Another analytical method, high pressure liquid chromatography (HPLC), has gained prominence in the last few years and has promise of becoming a widely used clinical laboratory tool not only for drug analysis, but many other biochemical laboratory procedures as well.¹

Of utmost importance in any analytical instrumental technique is the method of detection. Various chemical and physical properties have been used to detect chemical species. Among those commonly used are absorption of electromagnetic radiation in the infrared (IR), visible, and ultraviolet (UV) regions, thermal conductivity, refractive index, fluorescence, and detection of radioactive isotopes. Those commonly used with HPLC are UV and refractive index.

It was the purpose of the present study to show the feasibility of using HPLC as a chemical separating device and tritium exchange (TX), a radiochemical method (discussed in detail in Section IIA) of detection to identify and quantitate drugs of abuse, as they might be obtained after utilizing preliminary clean-up techniques on blood or urine samples. The process seemed to offer both a convenient method of separation and a capability of detecting a few parts-per-million or less concentration of drugs. If successful, a working unit was to be delivered to Midwest Research Institute for clinical trials.

SECTION II

TECHNICAL DISCUSSION

An experimental investigative program was undertaken to test the feasibility of, and construct breadboard instrumentation, using a combination of high pressure liquid chromatographic and tritium exchange techniques to separate, detect and quantitate common drugs of abuse and their metabolites as they might be extracted from clinical urine samples.

A. Background

1. High Pressure Liquid Chromatography

High pressure liquid chromatography (HPLC) is quickly becoming a common laboratory method for the separation, detection, identification and quantitative determination of material which are obtained as mixtures. The excellent book by Kirkland² clearly explains the theory and practice of this procedure.

One of the chief advantages of HPLC over gas chromatography (GC) is that it may be used to separate compounds of low volatility or compounds which are thermally unstable at temperatures required to reach high volatility. Drugs of abuse fall into one or both of these categories thereby making HPLC a preferred method of separation. Another advantage is the wide choice of liquid carriers available in HPLC as opposed to gaseous carriers in GC, thereby affording the practitioner an additional parameter to vary to achieve proper separation of components in a mixture. This advantage is important in the present case not only because of the large number of drugs and their metabolites which may be in the sample but also because of other possible substances which may be in the sample, even in trace quantities. However, one must be cognizant of the limitations placed upon the choice of carrier by such items as reaction with the sample to be separated or interference with the detector system. For example, carriers which absorb radiation in the ultraviolet region would not be used with a detector using this means of sample detection. In the present instance such considerations have placed severe restrictions not only on the choice of carrier but also upon trace impurities which the carrier might contain.

2. Tritium Exchange

The tritium exchange (TX) detection technique using a polymeric TX column substrate was initially developed by the Monsanto Company. Panametrics, Inc. is the licensee for this technology.

Tritium exchange is a process by which hydrogen atoms on molecules are replaced by tritium atoms, a radioactive isotope of hydrogen, thereby labeling the molecules and making them detectable by suitable radiation detectors. Tritium exchange is specific for compounds containing active^{*} hydrogen atoms. In the present instance the source of tritium atoms is a polymeric substrate which contains the repetitive unit $C_{14}H_{10}O_2N_2$, two of the hydrogens being active and having a statistical possibility of containing the tritium atoms to be exchanged. Molecules to be tritiated are brought into contact with the tritiated polymer.

Prior to being tritiated the polymeric substrate is loaded into a Teflon lined stainless steel tube approximately the size of a pen-light battery and, after appropriate Teflon filters are added to the ends of the tube, stainless steel valves are attached to both ends.

Activation of the substrate is accomplished by placing it in the presence of T_2O in which instance some of the active hydrogens on the substrate are replaced with tritium atoms. The extent of replacement is dependent upon the specific activity of the T_2O utilized for activation. The polymeric substrate is exposed to the T_2O until both substances have the same specific activity. The column so activated is termed the tritium exchange (TX) column (Fig. 1).

Compounds containing active hydrogen should quickly exchange these active hydrogens for tritium atoms when the compound is brought into contact with the activated substrate in the TX column. As in the substrate activation process the extent of exchange is limited to the point where both substances, the compound and the TX substrate, have the same specific activity.

The concentration of tritium atoms in the TX column effluent, which is proportional to the concentration of active hydrogen atoms,

*Active hydrogen as used in this report should be interpreted in the sense of capability of rapid exchange.

TRITIUM EXCHANGE COLUMN

FIG. 1

is determined by vaporizing the effluent (if it is not already in the gaseous state) and passing it through an ionization chamber (Fig. 2). The current produced, measured via an electrometer, is proportional to the concentration of tritium atoms in the ionization chamber. The absolute concentration of tritium in the ionization chamber (IC) is determined by precalibrating of the TX column-IC combination using known concentrations of water vapor in a nitrogen carrier stream. Sensitivities using newly prepared tritium exchange columns were in the order of 10^{-12} to 10^{-11} amp/ppm_v H₂O, which is in good agreement with theoretical calculations based upon ionization chamber geometry and the specific activity of the column activating supply (not the same for all columns).

Liquid scintillation counting, another means of radiation detection applicable in the present case, was not tried because other problems, not associated with the counting technique, could not be eliminated during the course of the study.

B. General Requirements

If feasibility was to be demonstrated, a number of items had to be accomplished during the investigation. These may be summarized as follows:

- (1) Demonstrate that drugs will be tritiated when brought into contact with the TX substrate.
- (2) Determine which liquid chromatographic system (column and carrier) would successfully separate a mixture of secobartital, methadone, morphine, and amphetamine which were chosen as the prototype drugs.
- (3) Properly treat the carrier liquid(s) to remove any impurities which contain active hydrogen atoms, i. e., water.
- (4) Determine which instrumental parameters and configurations would yield maximum sensitivity of detection.
- (5) Construct a working system for delivery to Midwest Research for clinical trials.

ION CHAMBER

FIG. 2

C. Tritiation of Drug

The Monsanto Company had indicated that tritium exchange would occur between the TX polymer and any compound containing an active hydrogen but the only compounds tested were water and ammonia in a system using a gas as a carrier. A gas flow system similar to that of Monsanto's has been used at Panametrics for continuous monitoring of water content in a gaseous stream.

Although there was no reason to doubt that tritium exchange would occur between the TX polymer and the drugs to be tested, the possibility did exist that exchange might be prevented by steric considerations since both the TX polymer and the drug molecules have complex structures. It was therefore deemed advisable to demonstrate exchange.

1. Experimental

A TX column was fitted with a syringe needle at one end and connected via its other end to a vacuum line and evacuated. A 0.50 ml sample of 1,2 $\text{C}_2\text{H}_4\text{Cl}_2$, previously dried over Drierite and placed into a septum sealed vial, was drawn up into the TX column via the syringe needle. The column was maintained at a temperature of 50°C and the 1,2 $\text{C}_2\text{H}_4\text{Cl}_2$ kept in the column for 20 minutes. The 1,2 $\text{C}_2\text{H}_4\text{Cl}_2$ was returned to the vial by overpressuring the TX column with prepurified nitrogen. During this operation the vial was maintained at liquid nitrogen temperature to prevent escape of tritiated compound via a "bleed" syringe needle placed through the vial's septum to permit free flow of the prepurified nitrogen. An appropriate size sample was removed from the vial and its tritium content determined using scintillation counting procedures.

The above procedure was repeated except that in this instance the sample consisted of 10.0 mg of secobarbital dissolved in 0.50 ml of the Drierite dried 1,2 $\text{C}_2\text{H}_4\text{Cl}_2$.

2. Results

The results of the experiments are summarized in Table I.

Taking into consideration the dilution multiple and difference in sample size counted, the experimental specific activity ratio (SAR) solution/solvent = 44.

TABLE I. Results of tritiation tests.

Sample	H ₂ O conc. via K. F. (μ g/ml)	H ₂ O conc. (ppm _v)	Drug conc. (ppm _v)	Tritium activity (disint. /min)
1, 2 C ₂ H ₄ Cl ₂	135	120	-	1.2×10^5 for 0.10 μ l
Secobarbital in 1, 2 C ₂ H ₄ Cl ₂	135	120 ^b	6.6×10^3	5.3×10^4 for 0.0010 μ l ^a

^a Actual sample delivered to scintillator = 1.00 μ l, but equivalent to 0.0010 μ l of original sample because of dilution.

^b A Karl Fischer titration on a 5mg sample of pure secobarbital showed no water present. Therefore water concentration in the solution is assumed the same as in the solvent.

3. Discussion

Since the secobarbital sample yielded a specific activity 44 times greater than that of the solvent there appears to be no doubt that the drug has been tritiated. However, based upon the relative concentrations of drug and water an SAR of 55 would be expected. There are two possible reasons for the experimental SAR to be lower than the theoretical SAR. First, there is a small quantity of water in the prepurified nitrogen stream used to empty the TX column in both instances. This water would have been tritiated and then collected along with the samples since the collection vials were cooled with liquid nitrogen. If it is assumed that this small concentration of water, w, was the same for both the samples then the theoretical SAR would be given by

$$\frac{6.7 \times 10^3 + w}{0.12 \times 10^3 + w}$$

Obviously, a small amount of tritiated water from the nitrogen would decrease the theoretical SAR. The water content of the N₂ is approximately 10 ppm_v and approximately 1 liter of N₂ was used per sample, thereby adding approximately 9 μ g of H₂O to each sample.

Second, there is reason to believe, both from our own experiments and from manufacturer's specifications, that the 1, 2 C₂H₄Cl₂ contains trace

1

amounts of active hydrogen containing substances other than water. The acidity of 1,2 C₂H₄Cl₂ is listed* as 0.001% HCl by weight. The choice of reporting the acidity as percent HCl is arbitrary. However, since HCl would be expected as a thermal or photochemical decomposition product of 1,2 C₂H₄Cl₂ we may assume, with some justification, that it is HCl. 0.001% HCl corresponds to 27 ppm_v. However, since HCl possesses one active hydrogen per molecule versus two for both the drug and water, the net effect would be 14 ppm_v. Considering this HCl impurity and the H₂O added via the N₂ in the theoretical SAR calculation yields a value of 48, in much closer agreement with the experimental value.

The experimental result showing that the drug was tritiated, does not prove that the drug directly exchanges with the TX substrate. Since there is some water in the sample it is possible that an exchange occurs with water as an intermediary.

Nevertheless, the drug is tritiated and, within experimental error, to the extent anticipated. There is no reason to believe that similar results would not be obtained if the other drugs in this study were tested in a similar manner.

D. Liquid Chromatographic System

The drugs of interest in this study are secobarbital, amphetamine, methadone and morphine. In our experiments we used the hydrochlorides of amphetamine and methadone. Although these compounds are dissimilar in structure they are all polar materials capable of significant dipole-dipole interaction and hydrogen bonding. To achieve a separation of a mixture of materials having such properties with HPLC it is customary to use a column containing a polar packing and a carrier liquid which is sufficiently polar to elute the sample in reasonable time while still permitting resolution of the components. In the present case, due to severe restrictions placed on the carrier (see below), other column-carrier combinations were also tried.

The choice of carrier liquid is severely limited by the requirements which it must meet. These are:

*MC/B Chemical Reference Manual, June 1973.

1. high polarity
2. no active hydrogen in the liquid or in any impurity it may contain
3. must be dryable to an extremely low water concentration
4. thermal stability without use of interfering inhibitors.
5. readily available in pure form.

The carrier must be polar in order to elute the drugs from a polar column. It cannot contain active hydrogen atoms in its molecular structure nor may there be impurities which contain active hydrogen atoms since these will be tritiated upon passing through the TX column thereby producing a high background signal, adversely affecting TX column life and producing large amounts of radioactive waste. This requirement alone eliminates from consideration most common polar solvents such as alcohols, aldehydes, amines, acids, and esters. The solvent must be dryable since water possesses active hydrogens. It must be thermally stable since decomposition or oxidation products may be undesirable impurities and it should not contain an inhibitor since these usually possess active hydrogen sites.* It should be readily available in pure form since once passed through the TX column it is not recyclable.

Taking into consideration the above restrictions while examining a table of "Solvent Properties of Chromatographic Interest"² leads one to consider three possible substances. These are, in increasing order of polarity, CH_2Cl_2 , 1,2 $\text{C}_2\text{H}_4\text{Cl}_2$, and CH_3CN .

*For example, one supplier's reagent grade 1,2 $\text{C}_2\text{H}_4\text{Cl}_2$ contains up to 5 ppm_w monomethylether of hydroquinone as an oxidation inhibitor. Being a UV absorber this inhibitor is not present in the supplier's spectral grade 1,2 $\text{C}_2\text{H}_4\text{Cl}_2$. The presence or absence of such inhibitors is not indicated on the reagent container nor in the solvent specifications in the catalog. The presence of such inhibitors in solvents implies that the oxidative decomposition of the solvents without added inhibitors would be objectionable. Therefore, it is quite conceivable that spectral grade solvents may contain objectionable decomposition products if the solvent is old or has been stored at elevated temperatures.

1. Experimental

The liquid chromatographic system was a Chromatec Model 3100-1 with 254 mm UV detector. The columns, all supplied by Chromatic, Inc., were 4 feet in length with 1/4" OD and 2.1 mm ID. The sampling system was of the stop-flow variety. Spectral grade carrier liquids were used as they came from the suppliers. No attempts were made to dry the carriers at this point in the experiment.

Samples of drugs were made by dissolving approximately 10 mg each of amphetamine · HCl, methadone · HCl, morphine, and secobarbital into individual 1.0 ml aliquots of Drierite dried 1,2 C₂H₄Cl₂. The morphine did not completely dissolve and the supernatant solution was used for test purposes. Unless otherwise indicated, 10 µl samples of the drug solutions were injected into the liquid chromatograph.

2. Results

Chromasep-S (silica adsorbent) column

Only one of the drugs, secobarbital, could be eluted from this column even when CH₃CN was used as the carrier. A 1,2 C₂H₄Cl₂/CH₃CN carrier in the ratio 1/2 by volume, and a flow rate of 2 ml/min produced the best characteristic secobarbital peak with a retention time of 2.5 minutes and a baseline peak width of 0.8 minutes. Pure 1,2 C₂H₄Cl₂ or CH₂Cl₂ would not elute secobarbital.

Chromasep RPP (polyamide bonded to silica microspheres) column

With either n-hexane or CH₂Cl₂ as the carrier, secobarbital, amphetamine and methadone all eluted on the solvent front. A sample of the morphine solution yielded a small peak (in comparison to the others) on the solvent front with CH₂Cl₂ as carrier. Since morphine did not dissolve to any great extent in 1,2 C₂H₄Cl₂, the small peak was probably morphine injected at a lower concentration than was used for the other drugs.

Chromasep RPV (reverse phase Vydac) column

Drugs eluted on the solvent front when n-hexane or 1,2 C₂H₄Cl₂ was used as carrier.

Chromasep SCV (Vydac) column

Secobarbital was eluted in 4.0 minutes with a baseline peak width of 2.7 minutes when the carrier, 1,2 $\text{C}_2\text{H}_4\text{Cl}_2$, had a flow rate of 1.1 ml/min. None of the other drugs eluted even with the column heated to 70°C.

3. Discussion

The Chromasep SCV (Vydac) column is silica coated on a solid sphere with the high energy adsorption sites of the silica being chemically blocked. This is similar to the Corasil II column used at Midwest Research Institute to obtain a separation of secobarbital, methadone and amphetamine using a 1/1 mixture of 1,2 $\text{C}_2\text{H}_4\text{Cl}_2$ and CH_3CN .³ Given that secobarbital eluted from the Vydac column in 4.0 minutes using 1,2 $\text{C}_2\text{H}_4\text{Cl}_2$ as a carrier whereas it eluted from the Corasil II column in approximately 10 minutes using 10% CH_3CN in 1,2 $\text{C}_2\text{H}_4\text{Cl}_2$ as carrier³ it would appear that a Vydac column would yield a better separation of the drugs and might even permit the elution of morphine if CH_3CN were used as a portion of the carrier. The addition of CH_3CN to the carrier was not pursued at Panametrics because experimental results from drying trials gave indications that CH_3CN could not be tolerated with the TX column in the system (see Section IIE).

It is not customary nor usually fruitful to attempt the separation of polar materials on nonpolar columns. However, such an attempt was made since, if successful, such a system might have permitted the use of a nonpolar carrier such as n-hexane which could be dried to a greater degree than polar solvents. These unsuccessful attempts were made with the Chromasep RPV column, having a packing of solid core material to which has been chemically bonded octadecyl groups via stable Si-C bonds, and the Chromasep RPP column, having a packing of polyamide bonded to silica microspheres.

Although expanding at a fast rate, the variety of liquid chromatographic column packings having chemically bonded surface phases is rather limited. However, packing materials with a wide range of properties may be made by coating a solid stationary phase with an appropriate liquid (liquid-liquid chromatography) such as β , β' -oxydipropionitrile or carbowax. Columns having such a packing could not be considered for use because they tend to "bleed" and are usually maintained by saturating the carrier with the coating material. This would interfere with our tritium exchange system.

Rather than make further separation trials it was decided to use the secobarbital peak obtained using the Chromasep SCV column in conjunction with 1, 2 $C_2H_4Cl_2$ as carrier to continue the project involving the TX system. If detection capabilities could be developed for this single component, separation of multicomponent mixtures could then be approached as a separate problem.

E. Drying of Carrier Liquids

When using any type of detection device one always strives to minimize or completely eliminate materials which would interfere by reacting with the detector in a manner similar to the substance being analyzed. In our case substances containing active hydrogen atoms in their molecular structure are objectionable because they would be tritiated when passing through the TX column and then detected in the ionization chamber. The most ubiquitous substance having active hydrogens is water and all the carriers used in this work have it as a "contaminant".

A high concentration of water is undesirable from a number of standpoints. A high background signal would be produced in the ionization chamber. Such a signal in itself is not objectionable since it may be electronically bucked-out but such a signal was, in our case, accompanied by a high noise level because of background instabilities. Furthermore, depending upon the sensitivity of the system, the ionization chamber detector might be close to saturation just by the tritiated water contaminant. For example, a newly made TX column will yield a sensitivity (ionization chamber current) of about 1×10^{-11} amp/ppm_v H_2O . Our ionization chambers saturate in the vicinity of 5×10^{-10} amp. Consequently, a carrier containing 50 ppm_v H_2O would saturate the detector.

Of equal importance is the life of the TX column. In addition, one must be cognizant of the radioactive waste problem. Therefore, it is necessary to dry the carrier liquid to a few ppm_v H_2O or better. In general, the more polar the liquid, the harder to remove water from it.

1. Experimental

A number of different drying agents and techniques were tried in an effort to achieve a minimum level of water contamination in the carrier liquids. Drying agents included P_2O_5 , Drierite ($CaSO_4$), molecular sieve 4A, and Aquasorb AR.TM

TMAquasorb is the Mallinckrodt Chemical Works trademark for its P_2O_5 -based indicating moisture absorbent.

The techniques employed may be placed into two general categories. The first is the batch category. Included are distillation, bubbling dry N_2 through the carrier, and mixing the carrier liquid and drying agent in a sealed flask or bottle. The second category is a flow system. Here the carrier liquid is passed through a column containing the drying agent or through a tube maintained at low temperatures.

The water concentrations were determined by using the Panametrics Al_2O_3 hygrometer. In a number of instances these determinations were confirmed by Karl Fischer titrations or could only be measured via Karl Fischer titration because of solute interaction with the Al_2O_3 sensor.

2. Results

The experimental techniques and results are summarized in Table II.

3. Discussion

The use of CH_3CN as a carrier would have been welcomed if it could have been sufficiently dried. However, the data indicate that this was not possible. The apparent increased conductivity of the hygrometer with increased depth in the CH_3CN -molecular sieve system is possibly due to slight dissolving of an ionizable impurity from the molecular sieve.

In both the n-hexane and 1,2 $C_2H_4Cl_2$ trials the Drierite batch + flow system yielded the driest material. The batch predrying no doubt removed a majority of the water, thus allowing the column to further dry the solvent while maintaining a long column life.

The long times required to achieve maximum dryness in the batch-flow system, 25 hours in the case of 1,2 $C_2H_4Cl_2$ and 13 hours in the case of n-hexane, should not be interpreted as an increasing effectiveness of the Drierite column with time. Rather, what probably occurred was that the solvent emerged immediately from the Drierite column at its maximum dryness but it took time to dry down the flow system between the Drierite column and the hygrometer. Most of the water no doubt originated in the liquid chromatographic columns, Vydac in the case of 1,2 $C_2H_4Cl_2$ and reversed-phase Vydac for n-hexane.

From the viewpoint of dryness, n-hexane would be preferred as the carrier. However, n-hexane could not produce the required chromatographic separation.

While the writing of this report was in progress further experiments were conducted on the aspect of drying the carrier liquid.

In these experiments, involving the 1,2 $C_2H_4Cl_2$, a 1/4" OD 10 ft column of molecular sieve 4A was placed in series with, and following, the 4 ft Drierite column (see Fig. 3A). Measurements of dryness were made using the Panametrics Al_2O_3 hygrometer as the liquid emerged from the L. C. pump drain, and aliquots were analyzed via Karl Fischer titration.

Both methods of analysis indicated a water concentration of 11 ppm_v. This is somewhat lower than the lowest value (18 ppm_v) shown in Table II when the Drierite column alone was used. Although still not sufficiently dry for our purposes, the experiment does show that it may be possible, using these or other drying agents, to lower the water concentration to a more acceptable level.

The drier liquid, when run through the TX system, still produced a high background signal and quickly contaminated the ionization chamber.

A gas chromatographic check of the 1,2 $C_2H_4Cl_2$ showed at least two impurities present with a total concentration of about 2×10^2 ppm on a carbon count basis.* No attempt was made to identify the impurities.

$$\text{* concentration} = \frac{\text{total area of impurity peaks}}{\text{total area of all peaks}} \times 10^6$$

TABLE II. Results of drying experiments.

Carrier Liquid	Drying Agent	Technique	Lowest H ₂ O Concentration (ppm _v)	Comments
CH ₃ CN	Drierite (indicating)	Batch - In sealed flask	Not determined	CH ₃ CN immediately dissolved the CoCl ₃ indicator off the Drierite and shorted out Al ₂ O ₃ probe.
	Drierite	Batch - In sealed flask	78	Karl Fischer titration
	Molecular sieve 4A	Batch - In sealed flask	Not determined	Upon standing without stirring the Al ₂ O ₃ probe showed greater H ₂ O concentration with increased depth of CH ₃ CN (closer to Drierite).
	P ₂ O ₅	Batch - In sealed flask	Not determined	CH ₃ CN takes on milky white appearance immediately upon being placed into P ₂ O ₅ . After 3 days a yellowish-orange material settled to bottom. Amount of P ₂ O ₅ definitely decreased.
1,2 C ₂ H ₄ Cl ₂	N ₂ bubbled through Dew pt. N ₂ = -70°C	Batch	110	45 minutes of bubbling at 500 ml/min. N ₂ .
	Drierite	Batch - After 3 changes of Drierite	32	No specific amount of Drierite or 1,2 C ₂ H ₄ Cl ₂ used. Volume of Drierite $\cong$ 0.1 volume of 1,2 C ₂ H ₄ Cl ₂ . Total time - few days.
	Drierite	Batch + Flow. Drierite column $\sim$ 4 ft long and 5/8" ID glass. (See Fig. 3A + added hygrometer.)	18	Level achieved after 25 hours of 1,2 C ₂ H ₄ Cl ₂ flow at rate of 1.5 ml/min.
	Molecular Sieve 4A	Batch - In sealed flask	120	After 40 hr. of contact.

TABLE II. Results of drying experiments (cont'd)

Carrier Liquid	Drying Agent	Technique	Lowest H ₂ O Concentration (ppm _v)	Comments
n-Hexane	Drierite	Batch - In sealed flask	8	Stood overnight. 10 ppm _v after 15 minutes. Second aliquot of Drierite does not lower H ₂ O conc.
	Aquasorb	Batch - In sealed flask	10	Stood overnight.
	Drierite	Batch - Vacuum Distillation with Drierite in Distillation column.	53	Water content high. Probably due to N ₂ bleed which was necessary.
	None	Cooling to -60°C in flow system.	44	
	Drierite	Batch + Flow Drierite column ~4 ft long and 5/8" ID glass. (See Fig. 3A + added hygrometer.)	4	Level achieved after 13 hours of n-hexane flow at rate ~2.5 ml/min (see Discussion).

F. Trials of Liquid Chromatographic-Tritium Exchange System

1. Experimental

The various component parts of the liquid chromatographic-tritium exchange system are shown in Fig. 3. These components were used (not necessarily all at once) in a variety of configurations and under a variety of conditions to determine feasibility and to try to alleviate the problems encountered.

The carrier drying-liquid chromatographic system is shown in Fig. 3A. The carrier drying component consisted of bottle I, bottle II, the Drierite column and the millipore filter. The use of two bottles permitted continuous operation of the system by isolating the dry reservoir (bottle II) from the atmosphere while permitting additional carrier to be added via batch dried solvent bottles (bottle I). Bottle II was periodically replenished with carrier from bottle I by opening the intervening valves and overpressuring bottle I to 20 psig with prepurified nitrogen. The 0.5 μ millipore filter was placed into the system to trap Drierite particles.

The TX column (Fig. 3B) was shown in detail in Fig. 1. The 1/8" SS Swagelok Tee (Fig. 3C) was used to inject samples at points after the LC column. The vaporizer (Fig. 3D) and pyrolyzer (Fig. 3E) were constructed of 1/8" SS tubing of convenient shape. The vaporizer was used to vaporize the carrier and the pyrolyzer was used in an attempt to rid the system of possible tritiated particulate contaminants (see Discussion). The ammonia purge (Fig. 3F) was inserted to try to minimize or eliminate tritium buildup in the ionization chamber. Figure 3G illustrates the ionization chamber-electrometer detector and the eluant collecting system and tritium trap. In the trap, any tritiated compounds which might vaporize would come into contact with the dampened cotton and exchange with the water. Any water vapor would be trapped by the Drierite filled tubes.

In the Results and Discussion, the various configurations used will be described by a series of letters corresponding to the letters associated with the components in Fig. 3. For example, A, B, D, G refers to a system consisting of the liquid chromatograph followed, in order, by the TX column, the vaporizer, and finally the detection and collection system. All components were connected with stainless steel Swagelok fittings.

A. CARRIER DRYING SYSTEM AND LIQUID CHROMATOGRAPH

LENGTH OF .25" S.S. TUBING SURROUNDED BY A HEAT SINK USED AS VAPORIZER. LENGTH AND SHAPE DEPENDENT ON COIL CONFIGURATION. ENCLOSED IN OVEN.

G. DETECTION AND COLLECTION SYSTEM

COMPONENTS OF LIQUID CHROMATOGRAPH AND TRITIUM EXCHANGE SYSTEM - ARROWS INDICATE FLOW

FIG. 3

2. Results and Discussion

The results were very disappointing though not completely unexpected given our inability to dry our carrier liquids beyond the points indicated. This yielded high background signals, sometimes higher than expected given the particular water content. A second significant problem, that of radiation contamination of the ionization chamber and access tubing, could not be eliminated. The contamination occurred quickly (within a few minutes) after the TX column was opened and carrier flow commenced. The two effects, that due to contamination and that due to water content in the carrier, yielded high backgrounds, in some instances approaching the ionization chamber saturation point. But, overriding all of the problems is the one item which was most disappointing, namely, that at no time did we observe a change in detector signal which could be attributed to an injected drug sample. At times, with the background and contamination at tolerable levels, a substantial increase in signal would be observed at the approximate time of drug elution. However, the signal would then remain at or near the higher level, presumably because of tritium contamination in the ionization chamber.

Various schemes and component configurations were tried in an attempt to minimize the background, noise and contamination, and to try to detect a signal from a drug injection. Among the configuration tried were:

A, C, D, B, F, G
A, C, D, B, F, E, G
A, C, D, B, E, G
A, B, D, G

Where the vaporizer, D, preceded the TX column, B, they were both maintained at a temperature exceeding the boiling point of the carrier liquid. With the reverse situation, B was maintained at 50°C and D at a temperature exceeding the carrier's boiling point. The temperature of the pyrolyzer, E, was 700°C. The temperature of the ionization chamber was maintained at a constant temperature in the range of 100-150°C.

The purpose of placing the Swagelok Tee, C, into the system was to permit the injection of a sample without incurring the dilution effect of the LC column. Still no drug was detected.

In theory, NH_3 should be capable of decontaminating the ionization chamber by tritium exchanging with a contaminant and then flowing

out. Yet, purging the ionization chamber with NH_3 after an experiment did not significantly lower the contamination level. In a similar manner NH_3 should be capable of preventing contamination when it is mixed into the TX column eluant prior to its entering the ionization chamber. This scheme did not succeed either, and in one instance, when combined with the pyrolyzer and 1,2 $\text{C}_2\text{H}_4\text{Cl}_2$ as carrier, yielded the expected result of producing NH_4Cl (probably tritiated) which precipitated onto the ionization chamber inner surfaces. The only way in which the ionization chamber could be decontaminated was to dismantle it, thoroughly clean it with chromic acid, and polish the inside surfaces. Ionization chambers made of either brass or stainless steel required the same treatment.

The purpose of the pyrolyzer (E) was to thermally degrade high molecular weight tritiated materials which might have been in the flow system. It was thought that such particles might be the source of ionization chamber contamination and would originate in the flow stream by the TX polymer slowly degrading because of radiation damage and/or some of the TX polymer dissolving in the carrier. The use of the pyrolyzer had no effect on the contamination problem. Weighing of the TX polymer before and after extensive use in a TX column did not show any loss in weight.

We cannot offer an explanation for the rapid contamination of the detection system nor for our inability to decrease the rate at which it occurred. In a similar system in our laboratory, used to measure H_2O in a nitrogen stream and calibrate TX columns, the contamination of the ionization chamber is of much lower level and gradually occurs over weeks of time. We must therefore conclude that the source of contamination must be the carrier.

Before proceeding to a consideration of other experiments it would be helpful to examine the detection problem quantitatively. Let us consider the conditions under which a definite secobarbital peak was eluted from the liquid chromatograph, namely, 1,2 $\text{C}_2\text{H}_4\text{Cl}_2$ as the carrier combined with the Chromasep SCV column. The peak eluted, as measured via UV detector, had a baseline peak width of 2.7 minutes with a carrier flow rate of 1.5 ml/min. The 10 μl secobarbital sample injected had a concentration of 10 $\mu\text{g}/\mu\text{l}$ for a total of 100 μg secobarbital. Symmetrically about the center of the emerging peak, half a peak width contains 0.75 of the secobarbital sample or 75 μg . If we now assume that the TX column and ionization chamber replace the UV detector and that there would be no peak broadening over that seen with the UV detector then this 75 μg of secobarbital is diluted (on average) in (0.5 peak) $(\frac{2.7 \text{ minutes}}{\text{peak}}) (1.5 \text{ ml/min}) = 2.0 \text{ ml}$ of 1,2 $\text{C}_2\text{H}_4\text{Cl}_2$ for

an average concentration of about 16 ppm_v secobarbital. The difficulty in detecting this concentration of secobarbital is quite apparent when one considers that the 1,2 C₂H₄Cl₂ contained 18 ppm_v H₂O which produced a high background, and a noise level which at times approached half the background. The situation becomes worse if one were also to consider other possible carrier impurities which contain active hydrogen atoms such as HCl.

We were quite aware of the above problem which was probably further aggravated in our experiments by mixing and dilution in the dead volume of the TX column when the sample passed through the column in the liquid state. To improve the situation experiments were conducted with a LC column-carrier combination which permitted sharp peak elution on the solvent front, thereby concentrating by about 10 fold the secobarbital entering the TX column. Also, injections were made via the Swagelok Tee, thus eliminating the LC column. Larger and more concentrated samples were injected, for example 20 µl containing 20 µg/µl secobarbital. No positive data resulted from these experiments although a single cause could not be identified.

Also, the experimental configuration where the vaporizer preceded the TX column would eliminate the TX column mixing-dilution effect by having the sample passing through in the gaseous state. This configuration did not yield positive results either. Again, the cause is unclear.

G. Construction of System for Clinical Trials

Given our inability to demonstrate feasibility, a working system for clinical trials was not constructed.

SECTION III

CONCLUSIONS AND RECOMMENDATIONS

The theory behind the investigation was and is sound. High pressure liquid chromatography is an excellent technique for separating mixtures of substances such as drugs of abuse from each other and from other constituents. Radiochemical measurement via tritium exchange and radiation determination should provide a high degree of detection sensitivity. But the severe constraints placed upon the choice of the HPLC system, and the problems encountered, particularly with respect to carrier properties and purity, and ionization chamber contamination, would appear to limit the use of such a technique even if feasibility could be demonstrated.

We do not doubt that the quickly burgeoning field of HPLC will produce methods capable of being used within the constraints of our system. We also believe that the problems of carrier purity and detector contamination could be overcome with further experimentation and refinement (see addendum). If and when suitable HPLC systems become available, it might prove advantageous to further pursue HPLC-TX techniques and try to overcome some of the problems, particularly that of tritium contamination of the ionization chamber.

REFERENCES

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