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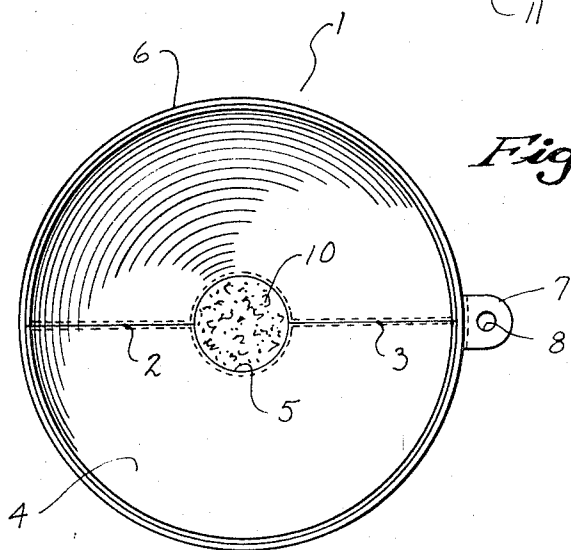
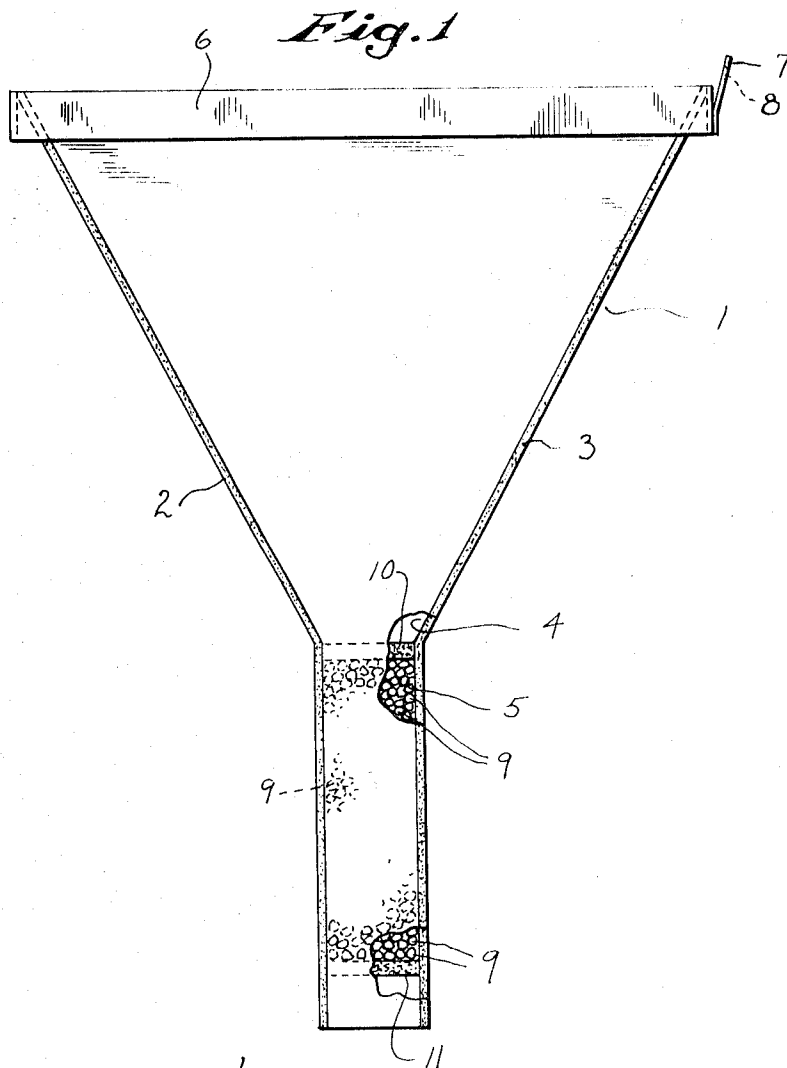
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ANALYSIS OF NARCOTICS AND AMPHETAMINES

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2 Sheets-Shoot 1



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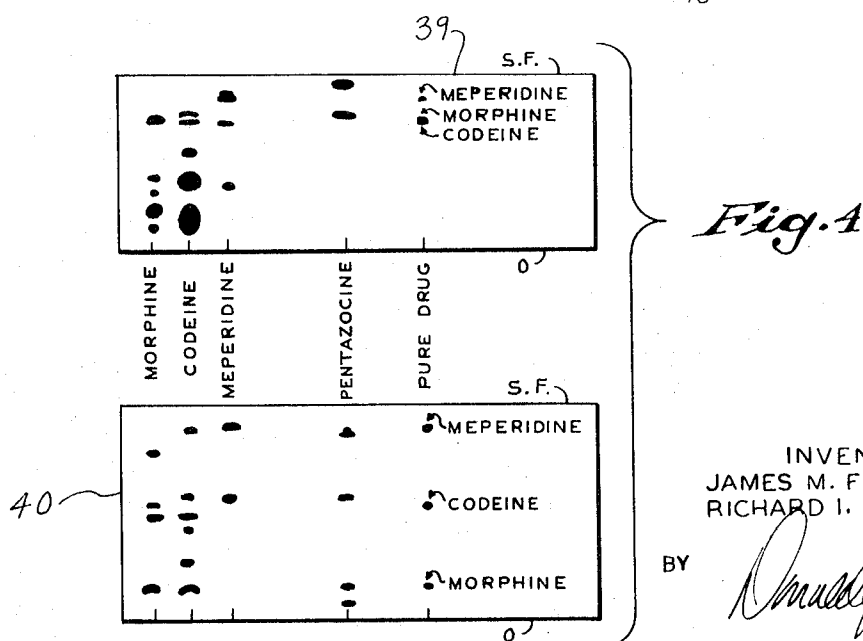
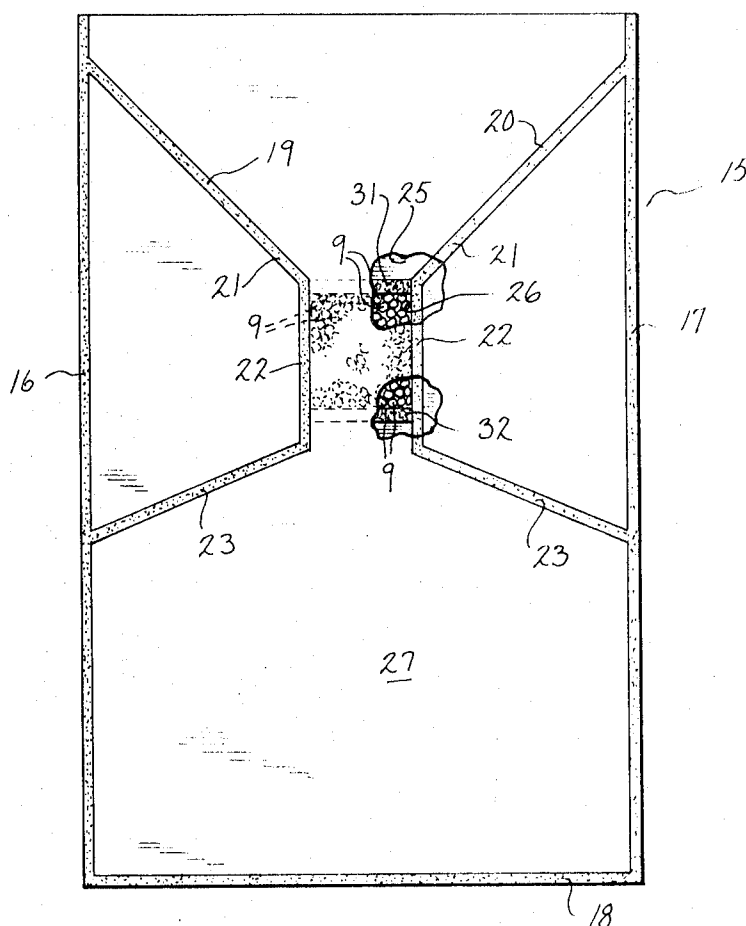
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Fig. 3



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ANALYSIS OF NARCOTICS AND AMPHETAMINES
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ABSTRACT OF THE DISCLOSURE

Apparatus and methods for the detection of narcotic analgesics and amphetamines which employ macroreticular heads of styrene-divinylbenzene copolymer to adsorb the narcotic analgesics and amphetamines from human urine in a nonionic mechanism.

BACKGROUND OF THE INVENTION

(1) Field

This invention relates to the field of urine analysis for the detection of narcotics, narcotic metabolites and amphetamines.

The invention described herein was made in the course of work under a grant or award from the Department of Health, Education, and Welfare.

(2) Prior art

One of the prior art methods for analyzing urine samples for the presence of narcotics and amphetamines is by extraction with organic solvents, but this system is time-consuming and cumbersome in its handling of solutions so that it is only suitable for research investigations instead of clinical work.

A more widely-practiced technique is the so-called Dole method which uses ion-exchange resins to separate the narcotics from a urine sample and is fully described in volume 198, Journal of the American Medical Association 115, Detection of Narcotic Drugs, Tranquilizers, Amphetamines, and Barbiturates in Urine, Dole et al. Briefly, the Dole method involves soaking a piece of cation-exchange paper in a diluted urine sample for 30 minutes or more, and extracting the components from the paper with three consecutive buffer-solvent systems having different pH values to separate the various drugs after which aliquots of the three solvent extracts are evaporated and the three residues are taken up with a suitable solvent for analysis by thin-layer or gas-liquid chromatography.

Although the Dole method has important advantages in comparison to the liquid extraction method, it also has several disadvantages which inhibit its utility for the rapid analysis of a large number of samples. It generally requires the use of bottles in which the cation-exchange paper can be immersed in the sample for the initial 30-minute extraction period and for the buffer-solvent extractions. Further a good deal of time is consumed in the three separate buffer-solvent extractions that must be performed on the paper to furnish a complete analysis, and additional time is needed for evaporation of the three solvent extracts.

Most users of the Dole method usually will employ only one of the buffer-solvent extractions (primarily the one specific for morphine and similar drugs), but even in this practice, the Dole method requires approximately 90 minutes to prepare one sample for chromatographic analysis after the initial immersion of the cation-exchange paper in the sample. A further disadvantage is the amount of space and equipment needed to conduct the test.

The present state of the art is such that there is a need for a rapid, accurate test method for narcotics and amphetamines and suitable apparatus by which it can be carried out in an economical fashion for analysis of a large number of samples; this need exists for observation of the therapeutic administration of narcotics and amphetamines and particularly the detection and treatment of drug abuse.

SUMMARY OF THE INVENTION

Our present invention is based upon the use of an insoluble solid having an open-cell microporous physical structure and a nonionic chemical composition to adsorb narcotics and amphetamines from urine samples. According to our method, a urine sample is passed through a short bed or column of the nonionic microporous solid material on which the drugs become adsorbed, the drugs are eluted from the microporous adsorbent and thereafter identified by any suitable means, preferably thin-layer chromatography. The apparatus, which is most suitably in the form of a plastic film structure, includes a first chamber through which the sample passes and a second chamber in fluid communication with the first chamber which contains the solid adsorbent material; after a sample has passed through the second chamber the narcotic analgesics and amphetamines are eluted from the adsorbent solid and analyzed.

We have found small beads of nonionic styrene-divinylbenzene copolymers having a porous macroreticular structure to be particularly effective for the adsorption of narcotic analgesics and amphetamines as described above.

Our technique provides a means to detect narcotics and amphetamines in urine that has a number of important advantages in comparison to the prior art methods discussed previously. The procedure to prepare a sample for analysis requires only about fifteen minutes as compared to the ninety minutes needed for the equivalent portion of the Dole method when using only a single buffer-solvent extraction. A single step elution is provided by the present invention which removes various narcotics from the urine instead of only removing a narrow band of extracts. The present test is sensitive without evaporation of the extracts before they are analyzed. Additionally, our method detects narcotic metabolites as well as the parent narcotic compounds without the need to use extra hydrolysis procedures, so that it is now possible to provide a "fingerprint" identification of a narcotic compound. A further important advantage is the provision of self-contained detection unit which gives the clinic or laboratory a pre-packaged apparatus suitable for carrying out our method in a convenient, economical and rapid manner.

One of the primary objects of this invention is to provide new methods and apparatus for the identification of narcotics and amphetamines in human urine; another is to provide a narcotic and amphetamine identification means that is sensitive but which can be performed rapidly; yet another is to provide an integrated unit which will detect narcotics and amphetamines. A more specific object is to provide the particular apparatus and methods hereinafter claimed.

DESCRIPTION OF THE DRAWINGS

The accompanying drawings depict several embodiments of the apparatus of this invention which are meant to illustrate, not limit, the invention inasmuch as it is anticipated that changes can be made in the illustrated embodiments by those skilled in the art which will remain within the true spirit and scope of the present invention. In the drawings:

FIG. 1 is a plan view of a pouch-like apparatus of this invention;

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FIG. 2 is a top view of the article of FIG. 1;

FIG. 3 is a plan view of another form of apparatus according to this invention; and

FIG. 4 is a plan view showing two thin-layer chromatograms of materials detected by this invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

(I) Description of method

The method of the present invention comprises, generally, the steps of (1) passing the urine sample through a small bed of macroporous beads of styrene-divinylbenene copolymer resin to adsorb narcotics and amphetamines from the sample, (2) eluting the narcotics and amphetamines from the adsorbent solids with a suitable polar organic solvent, and then (3) analyzing the eluate for the presence or identification of narcotics and amphetamines.

The styrene-divinylbenene copolymer resin is available commercially in the form of 20 to 50 mesh (U.S. Sieve Series) beads which have a high surface area due to their macroporous physical porosity; the beads are rinsed with 5% sodium chloride and 1% sodium carbonate prior to packaging to control bacteria and mold growth during storage. The resin beads were prepared for use by rinsing with distilled water and used in the form of a 60 mm. bed in a 130 x 11 mm. column in the examples set out below.

The adsorption step is carried out by passing the urine sample through the bed or column of the adsorbent resin at a fast flow rate. The method can be practiced with as little as 10 ml. of urine sample, although a sample as large as 250 ml. can also be used; the larger volume samples will increase the sensitivity of the test results, particularly if the drug is present at low dosage levels. After the last of the urine sample flows through the resin bed, the bed is washed with about 10 ml. of water. The adsorption step removes drugs and their metabolites from the urine, together with urinary pigments.

Elution of the adsorbed drug and metabolites from the copolymer resin is accomplished with a polar organic solvent such as methanol, ethanol or acetone. In the following examples, 10 ml. of methanol was used to elute the adsorbed materials. It was found that the adsorbed pigments provided a useful visual indicator in that they were adsorbed and eluted in the same fraction as the drugs and their metabolites so that collection of the highly colored fraction also yielded the desired narcotics and amphetamines. Using 10 ml. of solvent, the colored fraction forms about the first 5 to 7 ml. of eluate; in the following examples about 3 ml. of the most highly colored fraction of the adsorbed materials was collected for analysis.

The methanol fractions collected as described above were analyzed by thin-layer chromatography according to the following procedure. Sixty-microliter spots of the methanol fractions were applied to two thin-layer chromatogram sheets ("Gelman SG" silica gel impregnated glass microfibre sheets) and the spots were dried with a hot air blower. Twenty-microliter spots of urine standards were also applied to the chromatograms. The urine standards may comprise urine containing known drugs with metabolites, or urine containing the pure drug; however, in the latter instance, the "finger-printing" obtained from the presence of drug metabolites is lost, as described in detail hereinafter. The 60-microliter volume was applied in 3 portions, and the 20-microliter volume applied in one portion without undue spread of the spots. One sheet was developed in solvent system A (consisting of n-butanol, acetic acid and water in proportions of 35:3:10, v./v.) for 35 min. and the other in solvent system B (consisting of chloroform saturated with concentrated ammonium hydroxide) for 9 min. The developed chromatograms were dried in an oven at 80-90° C. The two TLC sheets were sprayed moderately with the iodoplatinate reagent for visualizing the drug and its metabolites as purple, gray,

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black, or pink spots on a light pink background. The iodoplatinate was prepared according to the directions of Cochin and Daly, vol. 18 *Experientia*, page 294 (1962) by adding 250 ml. of a 4% solution of potassium iodide to 10 ml. of a 10% solution of platinum chloride and diluting with water to 500 ml. If the spots faded, the TLC was sprayed again with iodoplatinate reagent. Solvent system A was designed primarily for separating polar metabolites of narcotics. System B separated the parent compounds; R_f of about 0.1, 0.4, and 0.9 were obtained for morphine, codeine and meperidine, respectively, and pentazocine was above codeine. Because the present method detects drug metabolites in addition to the parent drug itself, each drug will show a characteristic series of spots on the TLC sheets, particularly the sheets developed in solvent system A. Each dot of the chromatogram developed with solvent system A comprises a metabolite, not all of which are presently identified. Because most samples gave multiple spots, a "fingerprint" identification is possible by direct comparison of the pattern of spots obtained with an unknown urine sample to that obtained from the urine standards.

The following examples will further illustrate the present invention; in each example, the urine samples were coded so that the person conducting the analysis had no knowledge of the identity of the drug.

Example 1.—Thirty samples of urine from patients known to be receiving meperidine (including anileridine) at doses of 25, 50, 75 and 100 mg., im. were processed according to the method described above. The drug was correctly identified in 27 of the samples.

Example 2.—Thirty-four samples of urine from patients receiving codeine (including oxycodone) at doses of codeine phosphate of 30 or 60 mg., p.o., were tested. Twenty-nine samples were identified correctly.

Example 3.—Three samples of urine from patients receiving morphine sulfate at a dose of 10 mg., im. were tested. The drug was correctly identified in two of the samples.

Example 4.—The present invention is also useful for the detection and identification of amphetamine which, while not legally classified as a narcotic, is subject to drug abuse. Eight urine samples from patients receiving doses of 2.5, 5.0 and 10.0 mg. taken orally were processed by the method described above except that the methanol eluate was evaporated to a small volume in an oven at 90° C. The concentrated solution was spotted onto the same TLC paper and developed with solvent B, after which the chromatogram was sprayed with bromo cresol green (0.25 gm. in 100 ml. of methanol); the amphetamine appeared as a blue spot. All eight samples were correctly identified as containing amphetamine. Six control samples analyzed at the same time also were correctly identified as containing no amphetamine. The sensitivity of the procedure was 1 microgram of amphetamine per ml. of urine, using 50 ml. urine.

Example 5.—Thirty-nine urine samples from persons receiving pentazocine at 15, 25 and 30 mg. doses, im., were analyzed by the above method. Upon comparison of the TLC sheets to a pentazocine standard, the drug was correctly identified in twenty-seven of the samples. Although pentazocine is not presently considered a narcotic, it can produce drug dependency of the morphine-type and may have drug abuse potential, see Sandoval and Wang, *Tolerance and Dependence on Pentazocine*, vol. 280 *New England Journal of Medicine* 1391 (1969).

FIG. 4 illustrates the type of TLC "fingerprinting" obtained with the present invention. Chromatogram 39 depicts the type of spot pattern resulting upon development with solvent system A and chromatogram 40 showing that produced by development with solvent system B for Examples 1, 2, 3 and 5. The latter solvent system is primarily for identification of the parent compounds although it also yields some metabolites, while solvent system A is intended for identification of metabolites.

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The letter "O" designates the origin and the legend "S.F." indicates the solvent front. The set of chromatogram spots referred to as "pure drugs" were obtained from urine containing the respective drugs in the pure or unmetabolized form.

In each of the foregoing examples, the drugs which were tested were present in the samples at therapeutic dose levels, which represent relatively low quantities. When a drug is being taken in an abusive manner by a drug addict, it will be used in much greater quantities usually on the order of two to three times therapeutic levels. The ability of the present method to detect drugs when administered in small therapeutic amounts indicates it will be useful to detect and monitor drug addiction. The method is sensitive to about 0.2 microgram per ml. of urine for morphine and similar drugs, using 250 ml. of urine sample. As shown above, it is not necessary to evaporate the eluates, but evaporation can be used if so desired and the sensitivity will be increased. It should also be noted that heroin will analyze as morphine because of the manner in which it is metabolized in the body.

(II) Description of apparatus

FIGS. 1-3 illustrate two forms of apparatus according to the present invention.

FIG. 1 shows a flat funnel shaped pouch 1 formed of two superimposed sheets of flexible plastic film, such as polyvinyl chloride film, heat sealed together along edge seams 2 and 3 to form a container having a first chamber 4 and a second chamber 5. A stiffening member 6 is attached to the top edge of the pouch 1 and includes a tab 7 having an aperture 8 for hanging the pouch. The second chamber 5 contains a desired quantity of beads 9 of styrene-divinylbenzene resin of the type described above in the form of a small bed held in place by plugs 10 and 11 of glass wool or similar porous material. When the pouch 1 is to be used to test a urine sample, the pouch is opened to the rounded shape as shown in FIG. 2, the stiffener 6 serving to hold the pouch in this condition, and the sample is poured through the first chamber 4 to pass through the second chamber 5 for adsorption on the beads 9. After the sample has drained through completely, the adsorbent beads are eluted as described previously for analysis of the narcotics. The pouch can be stored flat prior to use and also can be folded flat after the sample has been processed. An advantage of the present invention is that the pouch will be dry after adsorption takes place which lends itself to relatively convenient handling.

FIG. 3 illustrates a second container according to the present invention comprising a pouch 15 formed of two sheets of material heat sealed together along side seams 16 and 17 and bottom seam 18 to form rectangular container. Heat seal seams 19 and 20 join the two sheets together near the top of the pouch, each comprising a first oblique seam portion 21 joined to a vertical seam portion 22 which in turn is connected to a second oblique seam portion 23. The pouch is thereby divided into a first chamber 25 which has a generally funnel-shape, a second smaller chamber 26 and a relatively large third chamber 27. A quantity of beads 9 is contained in the second chamber 26 and held in place by plugs 31 and 32 glass wool or similar porous or fibrous material. The top of the pouch 15 may be stiffened in the manner shown in FIGS. 1 and 2. After the sample passes through the first chamber 25 and is adsorbed on the resin contained in the second chamber, it is collected in the third chamber 27. The chamber 27 can include an outlet at the bottom for discharge of the sample; otherwise, the pouch 15 can be severed near the bottom of the second chamber 26 and the upper section preserved for analysis while the lower section is emptied or discarded; perforations can be formed partly across the pouch just above the third chamber to aid in its severance from the rest of the pouch.

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Plastic films such as polyvinyl chloride, nylon, polyethylene, polypropylene, etc., which can be sterilized are suitable for the pouches 1 and 15. The film may be inherently heat sealable or can include a functional heat seal coating; laminated, coated or monofilm sheet material may be used. The size of the several chambers will depend upon the quantity of sample likely to be processed and the quantity of adsorbent solids used in the second chamber. It is also desirable that the film material be capable of being written on so that each pouch can be suitably identified.

There has thus been described methods and apparatus for the detection and identification of narcotic analgesics and their metabolites and amphetamines in urine based upon the adsorption of such compounds on a specified class of solid adsorbents. The adsorbent for the practice of the present invention is to comprise a solid material having a nonionic chemical composition and a macroreticular physical structure having a high surface area. The adsorbents are in the form of beads, each bead comprising an agglomeration of a large number of very small microspheres to provide an open-cell porous structure. The adsorbed materials are collected generally within the surface areas of the adsorbent and can be readily eluted therefrom with suitable solvents. A specific class of compounds of this definition consists of macroreticular beads of styrene-divinylbenzene copolymer resins. These are commercially available, for example, from Rohm and Haas Company under the trade names Amberlite XAD-1, XAD-2 and XAD-4. These resins have an average pore diameter ranging from 50 to 205 angstrom units and a surface area of from 100 to 850 square meters/gram. The XAD-2 styrene-divinylbenzene copolymer resin used in the preceding examples had a surface area of 315 square meters/gram, an average pore diameter of 91 angstrom units, and a porosity of 0.43 ml. pore/ml. bead. The drug compounds are held by the adsorbent primarily by Van der Waals type forces. Adsorbent resins of this composition are also capable of adsorbing pigments from the urine sample so that it is possible for the technician performing the test to have a visual indication of the most suitable fraction to be eluted for analysis.

The present technique may be utilized to analyze for narcotic analgesics in general, particularly as defined under the Harrison Narcotic Act. These are largely morphine-like drugs including morphine derivatives and morphine surrogates. This will thereby encompass drugs and narcotics receiving wide therapeutic use and will also include drugs which are subject to drug abuse or addictive use. Amphetamines, including amphetamine and methamphetamine, also can be detected by the present method which is particularly important because of their widespread abusive use. Detection of drug metabolites with the present invention requires no extra steps such as hydrolysis or other techniques, and the concurrent adsorption of metabolites enables the use of a convenient fingerprinting system for identification of the drugs, thereby enhancing the accuracy of the final result.

The narcotic and amphetamine analysis set forth herein represents a test method which can be easily performed and is particularly useful when large numbers of urine samples are to be analyzed because it can be performed rapidly. For example, it is presently believed that a trained technician can analyze fifty urine samples in three hours with the present invention. Furthermore, the types of apparatus described herein provide self-contained test units which are convenient to store and use.

We claim:

1. As an article of manufacture, a container formed of plastic sheet material joined together to define a unit suitable for the analysis of narcotic analgesics and amphetamines in a urine sample comprising, in combination: a first chamber having an open top through which the urine sample can be introduced, and a second chamber connected to the first chamber,

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- a bed of a solid adsorbent comprising beads of styrene-divinylbenzene copolymer in a second chamber, said copolymer having a nonionic chemical composition and a macroreticular physical structure to adsorb narcotic analgesics and their metabolites and amphetamines from a urine sample which passes through the second chamber. 5
2. As an article of manufacture, a container formed of plastic sheet material joined together to define a unit suitable for the analysis of narcotic analgesics and amphetamines in a urine sample, comprising, in combination: 10
- a first chamber having an open top through which the urine sample can be introduced, and a second chamber connected to the first chamber,
- a bed of solid adsorbent comprising beads of styrene-divinylbenzene copolymer in the second chamber, said copolymer having a nonionic chemical composition and a macroreticular physical structure to adsorb narcotic analgesics and their metabolites and amphetamines from a urine sample which passes through the second chamber, and 15
- a third chamber connected to the second chamber to receive a urine sample after it has passed through the second chamber. 20
3. A method for the detection of narcotic analgesics and their metabolites and amphetamines contained in urine comprising the steps of: 25

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- (1) adsorbing the narcotic analgesics, their metabolites and amphetamines from a urine sample by passing the urine sample through a bed of solid beads of styrene-divinylbenzene copolymer having a chemically nonionic composition and a macroreticular physical structure, and
- (2) eluting the narcotic analgesics, metabolites and amphetamines from the adsorbent solid with a solvent for analysis of the eluate.
4. A method according to claim 3, including the steps of: 30
- adsorbing pigments from a urine sample along with the narcotics, metabolites and amphetamines, and eluting the colored fraction of the adsorbed materials for analysis.

References Cited

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