United States Patent [19]

Hamilton et al.

[11] **3,873,270**

[45] **Mar. 25, 1975**

[54] **DETERMINATION OF METHADONE IN BIOLOGICAL SPECIMENS**

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[73] Assignee: **The United States of America as represented by the Secretary of the Air Force,** Washington, D.C.

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[52] **U.S. Cl.** ..... **23/230 B,** 23/253 R

[51] **Int. Cl.. G01n 33/16,** G01n 21/24, G01n 21/34

[58] **Field of Search** ..... 23/230 R, 230 B

[56] **References Cited**

OTHER PUBLICATIONS

William P. Butler, "Methods of Analysis," IRS Publication, No. 341, 88–91, revised June 1967.

Clarke, "Isolation and Identification of Drugs," The Pharmaceutical Press, London, 1969, p. 408.

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[57] **ABSTRACT**

Methadone extracted from a biological specimen is oxidized to benzophenone by contacting same with a ceric sulfate-sulfuric acid solution. The benzophenone is extracted with a solvent, and the resulting solution is subjected to spectrophotometric analysis, thereby obtaining an ultraviolet (UV) absorption spectrum. The measured absorbance at a wavelength of 247 millimicrons (nm) is proportional to the concentration of benzophenone in the solution and concomitantly to the concentration of methadone in the biological sample.

15 Claims, 2 Drawing Figures

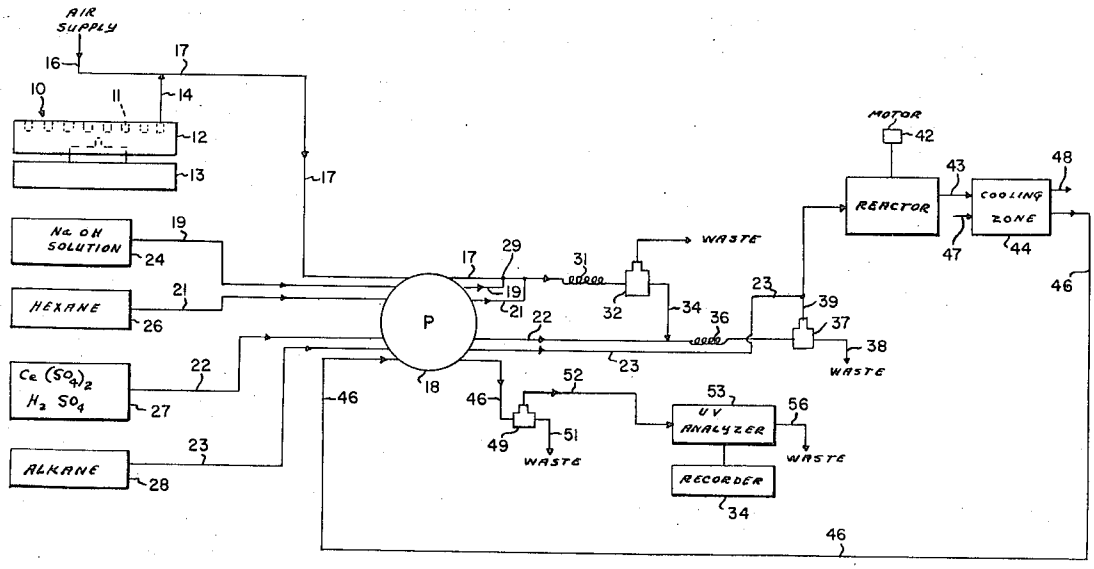
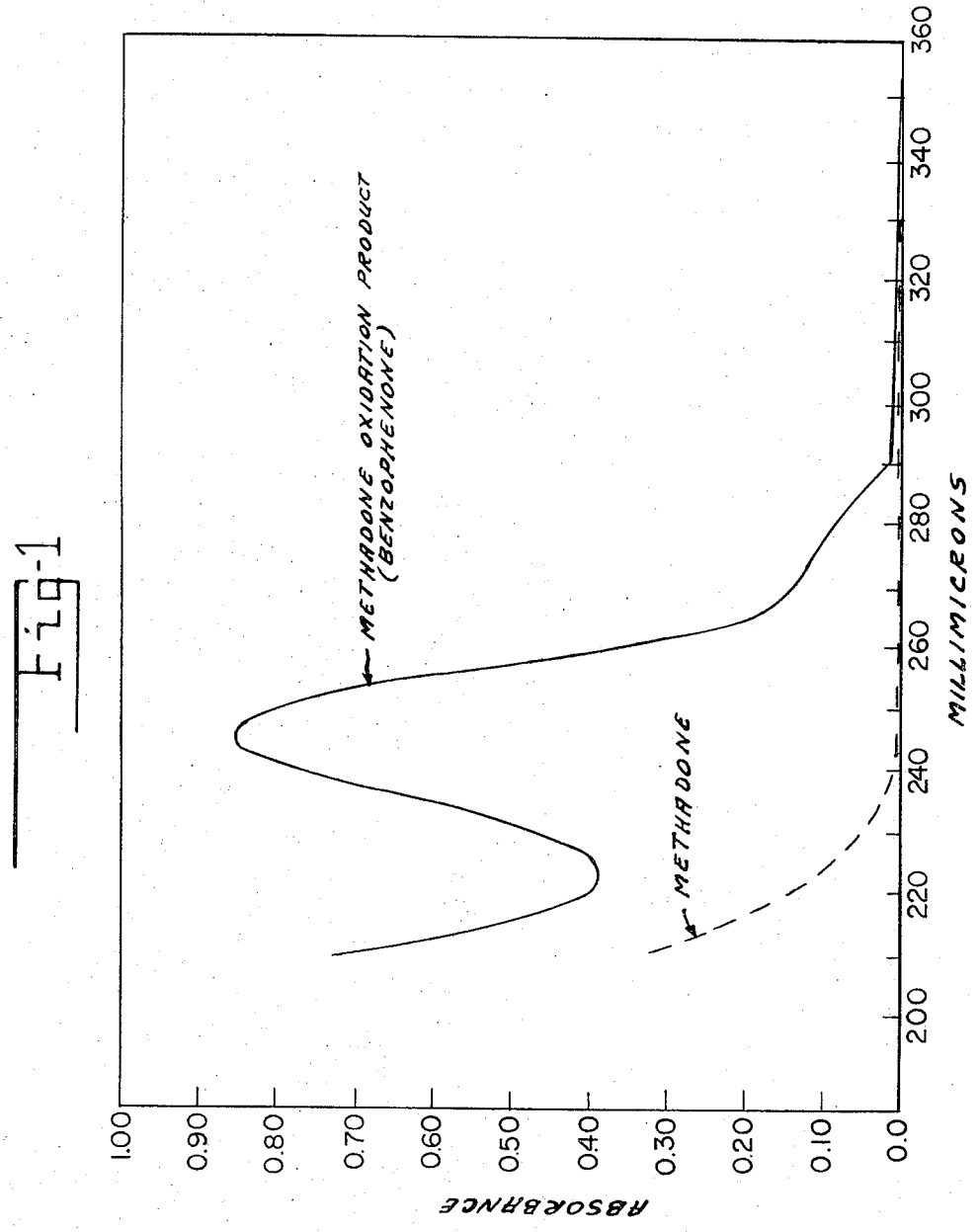


Fig-1


METHADONE OXIDATION PRODUCT
(BENZOPHENONE)
METHADONE
ABSORBANCE
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.0
200
220
240
260
280
300
320
340
360
MILLIMICRONS

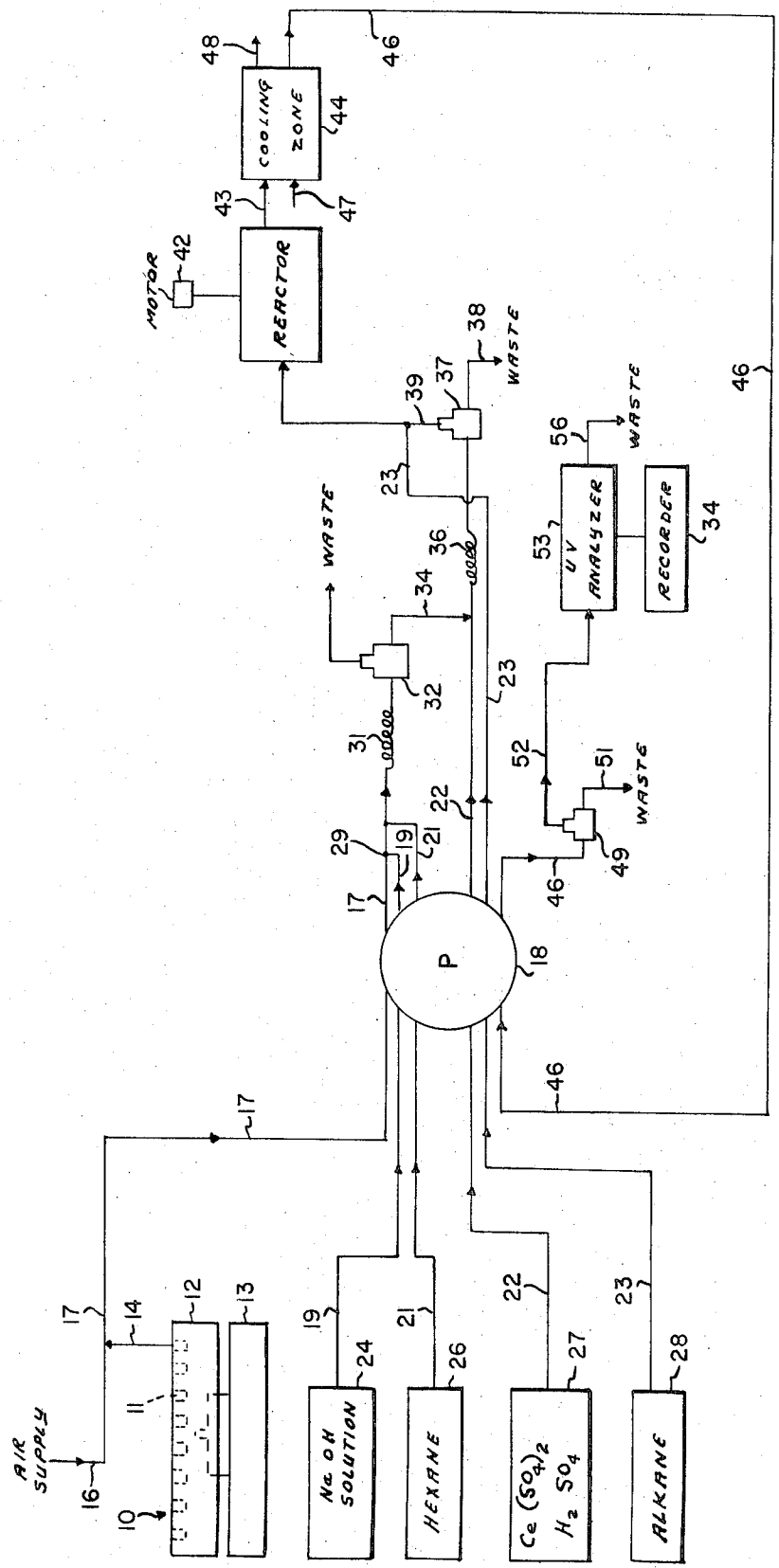

AIR SUPPLY
16
10
11
14
17
12
13
19
24
NaOH SOLUTION
21
26
HEXANE
22
27
Ce (SO4)2 H2 SO4
23
28
ALKANE
P
18
17
46
48
COOLING ZONE
44
43
47
MOTOR
42
REACTOR
38
WASTE
39
37
23
36
34
32
31
29
19
21
22
52
51
WASTE
49
53
UV ANALYZER
56
WASTE
RECORDER
34

Fig-2

DETERMINATION OF METHADONE IN BIOLOGICAL SPECIMENS

RIGHTS OF THE GOVERNMENT

The invention described herein may be manufactured and used by or for the Government of the United States for all governmental purposes without the payment of any royalty.

FIELD OF THE INVENTION

This invention relates to a method for determining the presence of as well as the concentration of methadone in biological specimens. In one aspect it relates to a method of methadone determination that is particulary suitable for adaption to an automated analytical procedure.

BACKGROUND OF THE INVENTION

Methadone, an antitussive and analgesic agent, has been used in recent years in the treatment of drug addicts. Several investigators have claimed that the methadone maintenance program is the preferred treatment for heroin addiction and have reported 75 percent success in achieving sustained rehabilitation.

As methadone maintenance programs grow in popularity and number, opportunities for abuse of methadone use will increase. It has been reported that the number of deaths resulting from methadone overdose has increased significantly since 1965, the year the maintenance programs were introduced. In describing a number of deaths resulting from methadone abuse, one investigator observed that individuals obtained large quantities of the drug by enrolling simultaneously in several programs.

The increased use of methadone accentuates the importance of an effective method for its quantitative determination in biological specimens. While spectrophotometric methods are available, sensitivity is less than satisfactory. Gas chromatographic and polarographic methods have been described, but the degree of technical proficiency required to analyze methadone by these methods renders them unacceptable to many laboratories. In the *Journal of Pharamaceutical Sciences*, 61, 1397–1400 (1972), Wallace et al describe an analytical method based upon the oxidation of methadone to benzophenone, a compound having a much greater absorptivity for ultraviolet radiation than methadone. However, the method disclosed has certain disadvantages, e.g., the formation of a second product during the oxidation that must be removed by extraction.

It is an object of this invention, therefore, to provide an improved method for the determination of methadone in biological specimens.

Another object of the invention is to provide a method of methadone determination that is adapted to automated procedures.

Other objects and advantages of the invention will become apparent to those skilled in the art upon consideration of the following disclosure and the drawing, in which:

FIG. 1 shows the UV absorption spectra of methadone and the methadone oxidation product (benzophenone); and

FIG. 2 is a flow diagram of an arrangement of apparatus for automatically analyzing for methadone in urine specimens.

SUMMARY OF THE INVENTION

The present invention resides in the discovery that methadone in in aqueous solution in the presence of an alkane can be readily oxidized to benzophenone by contacting same with ceric sulfate. By subjecting a solution of the benzophenone so obtained to spectrophotometric analysis, the absorbance of benzophenone is determined, thereby providing an indication of the amount of benzophenone, as well as the amount of methadone, that is present. Since benzophenone possesses a molar absorptivity approximately thirty-four times that of methadone, the present method provides a highly sensitive procedure for determining the presence and amount of methadone in biological specimens. Furthermore, the present method is a definite improvement over that disclosed in the Journal of Pharmaceutical Sciences in that a second product which must be removed, is not formed during the oxidation.

In a specific embodiment of the invention, methadone is extracted from a sample of urine or alkaline digested tissue by means of a measured amount of n-hexane. Thereafter, the amount of n-hexane recovered is determined and then extracted with a measured amount of dilute sulfuric acid. An aqueous acid layer containing methadone is recovered from this extraction. A mixture of the aqueous acid layer containing methadone, an alkane containing 7 to 12, inclusive, carbon atoms, and ceric sulfate, all in measured amounts, is refluxed for a period of time sufficient to oxidize the methadone to benzophenone. After separation from the reaction mixture, the alkane containing benzophenone is subjected to spectrophotometric analysis, and from the UV spectrum the absorbance of benzophenone is determined at 247 nm. The methadone concentration in a biological specimen can be readily determined from the following equation:

$$\frac{OD\ unk^{(1)}}{OD\ Std^{(3)}} \times \text{Conc. Std. in alkane (mcg/ml)} \times \frac{\text{ml hexane used in extraction}}{\text{ml hexane recovered}} \times \frac{\text{ml acid used in extraction}}{\text{ml acid used in reflux}} \times \frac{\text{ml alkane}}{\text{ml(g) specimen}} = \text{mcg/ml}^{(2)}$$

1. Optical density or absorbance.
2. Concentration of methadone.
3. For a standard, an aqueous methadone solution of known concentration is extracted with n-hexane; the n-hexane is extracted with sulfuric acid; an alkane and ceric sulfate are added to the acid containing methadone; and, after refluxing, the alkane containing benzophenone is analyzed to determine the absorbance of benzophenone.

In a particularly preferred embodiment, a solution of ceric sulfate in sulfuric acid is mixed with the n-hexane containing methadone. The aqueous acid extract that is recovered is then refluxed with the alkane so as to oxidize the methadone contained in the extract to benzophenone. The alkane containing benzophenone is then separated and analyzed as described above. This procedure is preferred because it permits the use of a pre-

mixed sulfuric acid-ceric sulfate solution, thereby eliminating the requirement of separate addition of the reagents with a concomitant saving in time and expense.

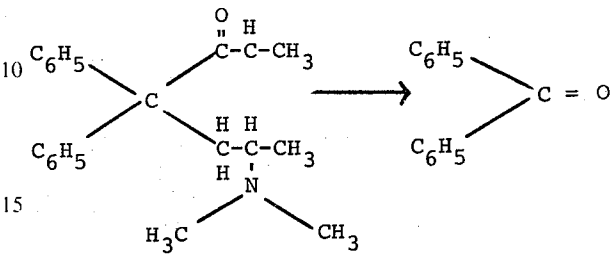
In the preliminary extraction of methadone from urine, a urine specimen, e.g., 6 to 20 milliliters (ml), is placed in a separator. The pH of the sample is then adjusted to 9 to 12.5 by the dropwise addition of sodium hydroxide, e.g., 5N sodium hydroxide. Several volumes of n-hexane, e.g., 2 to 10 volumes per volume of the specimen, are added to the specimen after which the resulting mixture is shaken vigorously for several minutes, e.g., from 2 to 5 minutes. Thereafter, the contents of the separator are allowed to settle, and the aqueous layer is discarded. The liquid remaining comprises n-hexane containing methadone.

In the preliminary extraction of methadone from tissue, the tissue specimens are alkaline digested to release protein-bound methadone. Thus, an amount of tissue, e.g., 5 to 15 grams, is combined with an aqueous potassium hydroxide solution, e.g., 5 to 20 ml of 30 percent potassium hydroxide solution. A flask containing the mixture is immersed in a bath of boiling water for about 10 to 30 minutes or until complete disintegration of the tissue is obtained. The resulting solution is then cooled to room temperature and transferred to a separator. Thereafter, its pH is adjusted to 9 to 12.5 by the dropwise addition of sodium hydroxide, e.g., 5N sodium hydroxide. After 3 to 10 volumes of n-hexane, based on the volume of the solution, have been added to the separator, the mixture is shaken for several minutes. The contents of the separator are then allowed to settle, and the aqueous layer is discarded. The liquid remaining comprises n-hexane containing methadone.

The extraction efficiency is optimal over the 9 to 12.5 pH interval, decreasing slightly from pH 9 to 7 and falling off more sharply thereafter. In strongly alkaline media (pH>13), methadone is reversably converted to a water-soluble form and extraction recoveries are negligible. In order to avoid emulsions, at least two volumes of hexane are utilized for each volume of urine. However, for maximum methadone recovery, 5 to 10 volumes of hexane are usually employed. For example, in a 3-minute extraction of 1 volume of urine with 5 volumes of hexane, a mean methadone recovery of 85 percent is obtained as contrasted to a 81 percent recovery using 2.5 volumes of hexane. In a preferred procedure, the combination of two successive extractions is employed, thereby providing a 95 percent recovery of methadone.

In the extraction of n-hexane, about 5 to 15 milliliters of dilute sulfuric acid, preferably 5 to 10 milliliters, are added to the n-hexane containing methadone. The molar concentration of the sulfuric acid is generally in the range of about 4.5 to 7.0, preferably from 5.0 to 6.0. After shaking the resulting mixture for about 2 to 5 minutes, it is allowed to settle and the aqueous acid layer containing methadone that forms is recovered. To the sulfuric acid containing methadone, there is then added about 2 to 15 milliliters of an alkane containing 7 to 12 carbon atoms and ceric sulfate. It is usually preferred to employ n-heptane as the alkane. The amount of ceric sulfate added generally falls in the range of about 200 to 300 milligrams.

After addition of the materials as described in the preceding paragraph, the mixture is refluxed for a period of about 20 to 60 minutes, preferably from about 25 to 40 minutes. The reaction mixture is continuously stirred during the reflux period. At the end of this period, the reaction mixture is cooled, e.g., to room temperature, and the hydrocarbon phase containing an oxidation product is separated from the aqueous acid phase. The product produced by the oxidization of methadone with ceric sulfate is benzophenone as depicted by the following equation:



The fact that the oxidation of methadone produces benzophenone has been established by the observation that the oxidation product and benzophenone exhibit identical ultraviolet and infrared spectra, gas chromatographic retention times, and thin layer chromatographic R_f values. The separated solution of the methadone oxidation product, i.e., benzophenone, is then analyzed spectrophotometrically by measuring the ultraviolet absorption at 247 millimicrons (nm). The measured absorbance of the benzophenone in the alkane is an indication of the amount of methadone present in the biological specimen. By conducting a series of runs in which absorbance values are obtained for specimens containing known amounts of methadone, a methadone concentration-absorbance curve can be readily plotted. The curve can then be used in determining the concentration of methadone in specimens containing unknown amounts merely by measuring the absorbance of the benzophenone and referring that value to the curve for a reading of the methadone concentration.

The effectiveness of the method of this invention resides in the enhanced sensitivity of the methadone oxidation product (benzophenone) to ultraviolet absorbance as compared to methadone per se. This increase in sensitivity is depicted graphically in FIG. 1 which shows the ultraviolet absorption spectra of methadone (20 mcg/ml 0.1 N HCl) and of the methadone oxidation product in n-heptane derived from a sample of equivalent concentration. The increase in molar absorptivity of benzophenone in n-heptane ($\epsilon = 18{,}173$, $\lambda = 247$ nm) over that of methadone in 0.1 N HCl ($\epsilon = 554$, $\lambda = 292$ nm) is approximately 34 times.

As described hereinabove, the n-hexane containing methadone is extracted with dilute sulfuric acid after which ceric sulfate and the alkane are added to the separated aqueous acid containing methadone. However, in a preferred procedure, a solution of ceric sulfate in sulfuric acid is added to the n-hexane containing methadone. The methadone is thereby extracted from the n-hexane, and an aqueous acid solution of methadone and ceric sulfate is separated, e.g., by decantation, from the n-hexane. The separated solution is then mixed with the alkane after which resulting mixture is refluxed as described hereinabove to obtain a solution of benzophenone. At the end of the reflux period, the separated alkane containing benzophenone is subjected to ultraviolet analysis.

Referring to FIG. 2 of the drawing, there is shown a flow diagram for automatically analyzing for the presence and amount of methadone contained in urine specimens. A sampler **10** has a plurality of sample cups or vials **11** disposed in a removable tray **12.** The tray is positioned on a turntable **13** which is controlled by a timer (not shown) so that it rotates according to a desired predetermined schedule. Before commencement of the operation sample cups **11** are filled with a measured amount, e.g., 5 milliliters, of urine which is to be analyzed for methadone. As the turntable rotates, line **14** under control of the timer alternately lowers into each of the cups. Air supplied through line **16** aspirates each urine specimen from its cup through line **14** and into line **17.** As a result of the described operation, each urine specimen is caused to flow sequentially through line **17** separated by a volume of air. Apparatus that can be advantageously employed in delivering the urine samples to line **17** in the described manner in the AutoAnalyzer sampler of Technicon, Inc.

Line **17** leads to and emerges from peristaltic pump **18** which propels the urine samples through the line by peristaltic motion. Such pumps are well known in the art and comprise rollers and a platen between which line **17** is disposed. Through the operation of the rollers acting on line **17,** which is in the form of a flexible tube, successive samples of the urine separated by air are pumped through line **17.** In like manner, flexible lines **19, 21, 22** and **23** connected, respectively, to vessels or containers **24, 26, 27** and **28,** are operatively associated with pump **18.** The pump operates to propel successive amounts of the NaOH solution, hexane, aqueous sulfuric acid solution of ceric sulfate, and an alkane, such as heptane, from their respective vessels and through their respective lines. The individual volumes and rates at which the several reagents flow through the lines can be readily controlled by adjusting the diameters of the several lines. In general, the amounts of reagents are controlled so that they fall in the range disclosed hereinabove. A peristaltic pump that can be advantageously used in propelling desired amounts of reagents through the lines is the AutoAnalyzer proportioning pump of Technicon, Inc.

For ease of understanding, in the ensuing discussion the continuous method will be described with relation to a single urine sample as it passes through the system. However, it is to be understood that each successive sample undergoes the same process steps. Also, after all of the urine specimens in the sample cups have been analyzed, tray **12** can be immediately replaced with a tray having sample cups containing additional urine specimens. Thus, the analytical procedure can be continued in an uninterrupted manner. Each urine specimen is appropriately marked for identification as is the corresponding ultraviolet specimen obtained for that specimen.

After leaving pump **18,** lines **17** and **19** are connected to one another, preferably by means of a mixing tee **29.** As a result, the urine specimen is mixed with the dilute sodium hydroxide solution in line **19.** The amount of sodium hydroxide solution mixed with the urine specimen is that which is sufficient to adjust its pH to about 9 to 12.5. Upon leaving pump **18,** line **21** is connected to line **17** downstream from mixing tee **29.** Hexane in line **21** is thereby introduced into the urine specimen in line **17.** In passing through mixing coil **31** positioned in line **17,** the urine specimen and hexane are thoroughly mixed, resulting in the transfer of methadone from the aqueous phase to the hydrocarbon phase. The mixture is then introduced into phase separator **32** wherein separation of the aqueous and hydrocarbon phases occurs. The aqueous phase is withdrawn from separator **32** through line **33** and discarded as waste material. The hydrocarbon phase containing methadone leaves the separator through line **34** which is connected to line **22.**

Upon entering line **22,** the hydrocarbon phase containing methadone mixes with a measured amount of aqueous sulfuric acid solution of ceric sulfate. As mentioned hereinbefore, the acid solution of ceric sulfate is withdrawn from vessel **27,** and measured amounts thereof are propelled through line **22** by the action of peristaltic pump **18.** The acid solution of ceric sulfate and the hexane containing methadone flow through mixing coil **36** positioned in line **22.** In flowing through mixing coil **36,** the materials are thoroughly mixed prior to introduction into phase separator **37.** During the mixing operation that occurs in the coil, the methadone transfers from the hydrocarbon phase to the aqueous acid phase.

In phase separator **37,** separation of the aqueous acid phase containing ceric sulfate and methadone and the hydrocarbon phase takes place. The hydrocarbon phase (n-hexane) is withdrawn from separator **37** through line **38** and disposed of as waste material. The aqueous acid phase containing ceric sulfate and methadone is removed from the separator through line **39** to which line **23** is connected. At the junction of lines **23** and **39,** an alkane (preferably n-heptane) flowing in line **23** mixes with the aqueous acid solution of ceric sulfate and methadone. As discussed above, the alkane is withdrawn from vessel **28,** and measured amounts thereof are propelled through line **23** by the action of peristaltic pump **18.**

The alkane-aqueous acid solution mixture is next introduced into reactor **41.** The reactor comprises a closed container having a tubular coil, preferably in the form of a helix and fabricated from glass, positioned therein. The coil is immersed in a heat exchange liquid, such as water or mineral oil, which is heated by a thermostatically controlled heating element submerged in the liquid. A stirrer operatively connected to motor **42** and immersed in the liquid provides means for circulating the liquid within the reactor. Line **39** is connected to one end of the helical coil within the reactor while the other end of the coil is connected to outlet line **43.** The coil has a diameter and a length such as to provide the desired residence time in the reactor. In its passage through the coil, the mixture is heated to a temperature in the range of about 90° to 100°C, at which temperature methadone present in the mixture is oxidized to benzophenone. A heating bath module sold by Technicon, Inc., can be used as the reactor in the present system.

The mixture containing benzophenone is withdrawn from the reactor through line **43** and introduced into cooling zone **44.** The cooling zone comprises a closed container having a tubular coil disposed therein. One end of the coil is attached to line **43** while the other end is connected to line **46.** Inlet line **47** and outlet line **48** provide means for circulating a coolant, such as ice water, through the container and around the coil disposed therein. After its passage through the coil, the mixture

is withdrawn from the cooling zone through line **46** at about room temperature.

Line **46** leads to and emerges from pump **18** which operates to propel the mixture into phase separator **49.** The line from the cooling zone is placed through pump **18** so as to provide a closed system in terms of fluid pressure and flow rate. In the phase separator, a hydrocarbon phase containing benzophenone and an aqueous acid phase separate from one another. The aqueous acid phase is recovered from the separator through line **51** and discarded as waste. The hydrocarbon phase containing benzophenone is removed from the separator through line **52** and passed into ultraviolet analyzer **53** for spectrophotometric analysis. Associated with the analyzer is a recorder **54** which records the ultraviolet spectrum of the solution, adjusted for blank in accordance with conventional analytical procedures, as it passes through the constant flow quartz cells of the analyzer. The absorption at **247** millimicrons provides a value which indicates the amount of methadone in the urine sample. The alkane containing benzophenone is withdrawn from analyzer **53** through line **56** and disposed of as waste material.

The system described above makes it possible to automatically screen a large number of urine specimens for methadone. As a result, a screening operation can be conducted with a great reduction in technical man-hours and expense.

A more complete understanding of the invention can be obtained by referring to the following illustrative examples which are not intended, however, to be unduly limitative of the invention.

EXAMPLE I

A series of runs was conducted in which 5 to 10 ml specimens of urine containing known amounts of methadone were analyzed in accordance with the method of this invention. In each run the specimen was transferred to a separatory funnel after which its pH was adjusted to 9–12 by dropwise addition of 5N sodium hydroxide. The specimen was then extracted into 50 ml of spectrograde n-hexane in a 3-minute manual extraction. The n-hexane layer containing methadone was removed, filtered, and the recovered volume recorded. Ten ml of 5.5 M sulfuric acid was added to the hexane, and the resulting mixture was shaken for 3 minutes. Nine ml of the aqueous acid layer, 5 ml of spectrograde n-heptane and 200–300 mg of anhydrous ceric sulfate were placed in a 250 ml round bottom flask which was attached to a water-cooled reflux condenser. The mixture was refluxed for 30 minutes with constant magnetic stirring and a high reflux rate of about 200 drops per minute.

At the end of the aforementioned period, the heptane containing methadone oxidation product (benzophenone) was read in a spectrophotometer at 215–360 nm against a similarly prepared n-heptane blank. (A Beckman DK–2A ratio-recording spectrophotometer with **10**nm cells was used for the ultraviolet absorption measurements.) Analysis at a single wavelength was achieved by determining the absorption at 247 nm. For a standard, 10 ml of a methadone solution containing 15 mcg/ml was extracted and its absorbance determined in a similar manner. The methadone concentration was determined from the equation set forth hereinabove and the results are set forth in Table I below.

TABLE I

Amount of methadone added to urine specimens, mcg/ml	Absorbance[1] at 247 nm	Absorbance Concentration	Methadone[2] Recovery, mcg/ml
2.5	0.229	0.092	2.16
5	0.466	0.093	4.40
10	0.875	0.088	8.27
20	1.772	0.089	16.74
30	2.720	0.092	25.98

(1)Each value represents the average of 6 determinations, adjusted for blank.
(2)Average percent recovery of methadone by extraction from urine specimens equals 85.5 ± 2.2 (standard deviation).

From the data in the foregoing table, it is seen that the conversion of methadone to benzophenone is linear. A graph can be plotted from the data (absorbance against methadone concentration) from which methadone concentrations in unknown urine specimens can be readily determined from absorbance values obtained by following the described procedure. It is to be understood that the same conditions used in obtaining the data for the graph should be followed in analyzing the unknown specimens.

EXAMPLE II

A series of runs was conducted in which urine specimens containing added methadone at concentrations of 1.25, 2.50 and 5.0 mg % were analyzed. The urine specimens were each manually extracted into n-hexane and back into an oxidation medium consisting of a solution of ceric sulfate in 5.5 M sulfuric acid. Five ml aliquots of the oxidation medium containing methadone were then aspirated with 6 ml aliquots of dodecane through helical coils of a heating bath maintained at 90°C. After cooling, the dodecane containing the methadone oxidation product (benzophenone) was separated, and the absorbance of the benzophenone, adjusted for blank, was measured at 247 nm. The results of the runs are shown below in Table II.

TABLE II

Concentration of methadone in urine specimens, mg%	Absorbance at 247 nm	Absorbance/ Concentration
1.25	0.20	.16
2.50	0.45	.18
5.00	0.78	.16

From the data in the foregoing table, it is seen that the conversion of methadone to benzophenone is linear. On the basis of the data, an absorbance-methadone concentration graph can be plotted from which methadone concentrations in unknown urine specimens can be obtained by following the procedure described in this example. The example demonstrates the feasibility of using an automated system as depicted in FIG. **2** of

the drawing in analyzing for methadone in biological specimens.

As will be evident to those skilled in the art, modifications of the present invention can be made in view of the foregoing disclosure without departing from the spirit and scope of the invention.

We claim:

1. A method for determining the presence and concentration of methadone in a biological specimen which comprises extracting methadone from a biological specimen; mixing the extracted methadone with an alkane and ceric sulfate; refluxing the mixture for a period of time sufficient to oxidize any methadone present to benzophenone; separating from the mixture a solution of the benzophenone in the alkane; and subjecting the separated solution to spectrophotometric analysis, thereby obtaining an ultraviolet spectrum of the benzophenone.

2. A method according to claim **1** in which methadone is extracted from the biological specimen with n-hexane; the methadone is extracted from the n-hexane with dilute sulfuric acid; and the alkane and ceric sulfate are mixed with the dilute sulfuric acid containing methadone.

3. The method according to claim **2** in which the biological specimen is a urine specimen and the pH of the specimen is adjusted to **9** to **12.5** prior to extraction with n-hexane.

4. The method according to claim **2** in which the biological specimen is an alkaline digested tissue specimen and the pH of the specimen is adjusted to 9 to 12.5 prior to extraction with n-hexane.

5. The method according to claim **2** in which the alkane contains 7 to 12, inclusive, carbon atoms.

6. The method according to claim **5** in which the alkane is n-heptane.

7. The method according to claim **5** in which the alkane is dodecane.

8. The method according to claim **2** in which the absorbance of the benzophenone is determined at 247 millimicrons.

9. The method according to claim **3** in which about 2 to 10 volumes of n-hexane per volume of urine specimen are added thereto; the resulting mixture is shaken for about 2 to 5 minutes; and, after settling, n-hexane containing methadone is recovered.

10. The method according to claim **4** in which about 5 to 15 grams of a tissue specimen is combined in a flask with 5 to 20 milliliters of aqueous potassium hydroxide; the flask is immersed in a bath of boiling water for a period of time sufficient to cause disintegration of the tissue; after cooling the resulting solution, its pH is adjusted to 9 to 12.5; about 3 to 10 volumes of n-hexane per volume of the solution are added thereto; the resulting mixture is shaken for about 2 to 5 minutes; and, after settling, n-hexane containing methadone is recovered.

11. The method according to claim **9** in which about 5 to 15 milliliters of sulfuric acid are added to the n-hexane containing methadone, the molar concentration of the sulfuric acid ranging from about 4.5 to 7.0; the resulting mixture is shaken for about 2 to 5 minutes; after settling, the sulfuric acid containing methadone is recovered; and about 5 to 15 milliliters of the alkane and about 200 to 300 milligrams of the ceric sulfate are mixed with the sulfuric acid containing methadone.

12. The method according to claim **1** in which methadone is extracted from the biological specimen with n-hexane; a solution of ceric sulfate in dilute sulfuric acid is mixed with the n-hexane containing methadone; an aqueous acid solution of methadone and ceric sulfate is separated from the mixture; and the separated solution is mixed with the alkane.

13. The method according to claim **12** in which the mixture of separated solution and alkane is heated at a temperature ranging from about 90° to 97°C for a period of time sufficient to oxidizing any methadone present to benzophenone.

14. A system for continuously analyzing for the presence of methadone in urine samples which comprises, in combination, a plurality of sample cups; means for sequentially aspirating urine from the sample cups into a first tube; an alkaline solution tank; a second tube connected to the alkaline solution tank; a hexane tank; a third tube connected to the hexane tank; a ceric sulfate-sulfuric acid solution tank; a fourth tube connected to the ceric sulfate-sulfuric acid solution tank; an alkane tank; a fifth tube connected to the alkane tank; a peristaltic pump, the first, second, third, fourth and fifth tubes being operatively associated with and extending through the pump and at least those portions of the tubes positioned in the pump being flexible tubing; an extension of the first tube leading from the pump to a first liquid phase separator; an extension of the second tube connected to the extension of the first tube; an extension of the third tube connected to the extension of the first tube at a location downstream from the connection of the extension of the second tube to the extension of the first tube; a mixing means positioned in the extension of the first tube downstream from the connection of the extension of the third tube to the extension of the first tube; a first outlet line for removing a first liquid phase from the first liquid phase separator; a second outlet line for removing a second liquid phase from the first liquid phase separator; an extension of the fourth tube leading from the pump to a second liquid phase separator, the second outlet line from the first liquid phase separator being connected to the extension of the fourth tube; a mixing means positioned in the extension of the fourth tube downstream from its connection with the second outlet line from the first liquid phase separator; a first outlet line for removing a first liquid phase from the second liquid phase separator; a second outlet line for removing a second liquid phase from the second liquid phase separator; an extension of the fifth tube leading from the pump and connected to the first outlet line from the second liquid phase separator; a reactor; an inlet line connected to the reactor and to the connection of the extension of the fifth tube and the first outlet line; an outlet line connected to the reactor; a cooling means having the outlet line from the reactor connected thereto; an outlet line connected to the cooling means and leading to the pump, the outlet line being operatively associated with and extending through the pump and at least the portion of the line positioned in the pump being flexible tubing; a third liquid phase separator having an extension of the outlet line leading from the pump connected thereto; a first outlet line for removing a first liquid phase from the third liquid phase separator; a second outlet line for removing a second liquid phase from the third liquid phase separator; and an ultraviolet analyzer having the first outlet line from the third liquid phase separator connected thereto.

15. The system of claim **14** in which a recorder is operatively connected to the ultraviolet analyzer.

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