Description

This invention relates to analytical systems and methods for biological fluids such as serum and urine. In particular, this invention relates to chromatographic systems for drug analyses in biological fluids.

A wide range of analytical methods are known for the detection of toxic drugs in serum or urine. The most common techniques are those involving immunological interactions and those involving chromatographic separation. Immunological techniques directed at single species can provide highly accurate information regarding the presence and amount of the species in question. When a single immunological assay is used for multiple drug detection, it will generally detect only drugs of a particular class, and will not provide identification of the specific drugs which are present or their concentrations. Chromatographic techniques, including thin-layer chromatography, high performance liquid chromatography and gas chromatography, may permit detection of a multitude of drugs at the same time, but generally require extensive sample preparation and a total analysis time of 1-2 hours. Neither immunological nor chromatographic techniques as presently known are useful for rapidly analyzing wide ranges of drugs.

The present invention provides a chromatographic system and method which combines several unique features permitting a broad spectrum drug analysis by isocratic separation in an unusually short period of time.

The invention provides a system for analyzing a biological sample for the presence of drugs in the form of anions, bases and neutral compounds, in which system the sample is carried by a liquid carrier through means which remove from the sample substantially all of the unwanted components i.e. inorganic salts and proteins. The sample in the carrier then passes to a combination of analytical columns comprising an ion exchange medium, a reversed phase medium and a cation exchange medium. Finally the components of the sample emerging from the combination are detected.

In another aspect, the invention provides a method for analyzing a biological sample for the presence of drugs in which inorganic salts and proteins are removed from the sample which then passes through an anion exchange medium, a reversed phase medium and a cation exchange medium, in that order, before the components of the sample are detected after they emerge from the cation exchange medium.

The invention may provide an automated system which combines detection and scanning elements with a library of known spectra and retention times, to identify and quantify each component as it emerges from the column system fully separated from the other drugs originally present in the test sample. Still further, the invention may provide a system which combines column reconditioning features with its chromatographic functions so that portions of the system may be reconditioned or regenerated for subsequent test samples while other portions of the system are in use performing the separation of a sample already injected. The invention may also provide for the automatic injection of a multitude of test samples in sequence, with full system regeneration and conditioning in between each sample.

By virtue of these unique features, the invention provides for the analysis of any liquid test sample to identify and quantify a large majority of the several hundred most frequently prescribed drugs, as well as all drugs commonly analyzed by liquid chromatography. In particular, the system may allow for the highly specific separation and detection of four classes of basic drugs -- benzodiazepines, amphetamines, tricyclic antidepressants and opiates -- while grouping these drugs according to class. The system may further allow for the separation and detection of additional drugs of various types, including antihistamines, phenothiazines and barbiturates. As indicated above, the system lends itself well to automation, and as a result, full analyses can be obtained unattended in an unusually short period of time -- substantially less than 1 hour, and in many cases on the order of 15 minutes.

The separation media used in the present invention may be placed in a column arrangement in such a manner that separations of certain classes of drugs are performed primarily on a single column. In particular, the system can be arranged such that the anion exchange medium selectively retains hydrophobic anions and a major portion of neutral compounds, while the separation of benzodiazepines (and other weak bases) and hydrophobic bases and neutrals occurs on the reversed phase column. The cation exchange column provides for the separation of basic compounds, including further separation of those separated on the reversed phase column.

To further categorize these columns and how they are used: the anion exchange column is preferably selected and used in such a manner that it causes substantially no retention of either bases or very hydrophilic neutral compounds, although a slight retention of weak bases (e.g. benzodiazepines) is permissible. The separation of barbiturates and other weak acids may occur on this column.

The reversed phase column may be selected and used in such a manner as to cause substantially no retention of hydrophilic bases, barbiturates or other anions including those which may have passed through the anion exchange medium without retention.

Likewise, the cation exchange column may be selected and used in such a manner that there is substantially no retention of anions and barbiturates. A slight retention of neutral compounds is permissible in this column.

The anion exchange medium is preferably a polymeric resin having quaternary ammonium functional sites. A particularly effective polymer is styrene-divinylbenzene, and the functional sites are preferably tetra-(lower alkyl) ammonium moieties. A product which is particularly effective and commercially available as of the filing date of this specification is AMINEX® A-28 resin, supplied by Bio-Rad Laboratories. Hercules, California, which is an HPLC grade strongly basic anion exchange resin. with tetramethylammonium functional groups on an 8% cross-linked styrene-divinylbenzene matrix, with an average particle size of about 11 microns.

The reversed phase column may be a derivatized silica, preferably one bearing alkyl functional groups. Alkyl groups of choice are those containing alkyl chains of 6 carbon atoms or more, preferably from about 6 to about 18. In particularly preferred embodiments, the functional groups are attached by bonding the silica to a dimethylalkylsilane, in which the alkyl group is that referred to above. Silicas of this description are readily commercially available. In further preferred embodiments, the carbon loading of the silica, i.e., the carbon atom content in weight percent, ranges from about 6% to about 12%, with about 8% to about 10% especially preferred. A commercially available product which has been found to be effective is MOS-HYPERSIL®, a dimethyloctylsilane bonded to silica with a carbon loading of 9%, obtained from Shandon Scientific Ltd., distributed by Keystone Scientific, State College, Pennsylvania. The average particle size is approximately 5 microns and average pore size is approximately 120 Angstroms. For bonded silicas in general for this application, pore sizes of 50 to 120 Angstroms may be used.

A typical cation exchange medium is underivatized silica, widely available commercially. One example of a commercial product effective for this medium is ADSORBOSPHERE®, available from Alltech Associates. Deerfield, Illinois, consisting of an underivatized silica with an average particle size of 5 microns, a pore size ranging from 50 to 80 Angstroms, and a surface area exceeding 350 m^2/g.

By selection of the lengths of each of these columns, one can minimize or substantially eliminate interference between emerging peaks, and minimize the analysis time as well. In general, and particularly with the preferred column packings described above, minimal peak interference may be achieved with an anion exchange column ranging from about 10 mm to about 30 mm in length, a reversed phase column of about 10 mm to about 50 mm in length, and a cation exchange column of about 100 mm to about 250 mm in length.

The precolumn may be a polymeric resin of hydrophobic character. A preferred example is styrene-divinylbenzene, although other resins of similar character may be used. Resins of this type are widely available commercially, one example being a product designated PRP-1, a hydrophobic styrene-divinylbenzene copolymer having a particle size of 12-20 microns, available from Hamilton Co., Reno, Nevada. Typical column lengths range from about 10 mm to about 30 mm.

Test samples may be applied to the precolumn by a buffer solution having a pH of 7.5 or greater, preferably from about 7.5 to about 9.0. One example of such a buffer system is a 0.1% aqueous solution of potassium borate with a pH of approximately 8.0. This buffer solution may be used as a carrier solution to apply the samples to the column, and also as a purging solution to purge the column once the sample is applied, to remove from the sample those species whose detection by the system is not desired. This may be achieved by passing excess buffer solution through the sample-impregnated column, preferably in both directions. Species removed include inorganic salts, proteins, peptides and hydrophilic anions.

For chromatographic separations in the analytical columns, the carrier liquid preferably contains acetonitrile. The acetonitrile content may vary, and will generally fall within the range of about 10% to about 50% by volume. Higher acetonitrile concentrations within this range are effective to move components through the columns more rapidly than lower concentrations. For example, concentrations of about 40% by volume or above may be used to dislodge the components from the precolumn after the purging of unwanted species has been completed. Concentrations on the order of 35%, 30% and lower may likewise be used on the analytical columns to separate the drugs within each of the various classes. Carrier liquids similar to these but with acetonitrile concentrations higher than the above-indicated range are effective as wash solutions to recondition the columns after use and to thereby prepare them for the next sample.

Preferred carrier liquids contain additional agents for controlling the retention and/or selectivity of certain drug classes on particular columns, either to enhance separation or to shorten elution times. In particular, long chain alkylamines, preferably those having carbon chains of 6 carbons or greater, may be used to accelerate the elution of certain classes such as tricyclic antidepressants from the reversed phase column. A particularly effective example is dimethyl octyl amine. The concentration of this additive may vary, but will generally fall within the range of about 0.001% to about 0.05% (volume basis), with preferred

amount within the range of about 30 to about 300 microliters per liter of carrier liquid.

Likewise, retention time and selectivity on the cation exchange column may be controlled by inclusion of a quaternary amine in the carrier liquid. Again, concentrations may vary, although the amount will generally fall within the range of about 0.002 M to about 0.05 M. Typical quaternary amines are tetraalkylammonium hydroxides and halides. Examples are tetrabutylammonium hydroxide, tetraethylammonium hydroxide, and tetramethylammonium chloride. In preferred embodiments, the concentration ranges from about 0.1 to about 2.0 grams per liter of carrier liquid.

The carrier liquid also preferably includes a water-soluble organic solvent combined with an aqueous buffer solution. The pH is preferably from about 6.0 to about 7.5.

A convenient way of varying the acetonitrile content is by the use of stock solutions and a mixing device for combining the stock solutions in controlled but variable proportions. For example, a combination of two stock liquids may be used, the first being primarily acetonitrile, at least about 75% by volume, preferably 100%, and the second containing all of the other components, including the long chain alkylamine, the quaternary amine, the buffer and the solvent. In preferred embodiments, the alkylamine will be present in the second solution at a concentration of about 0.001% to about 0.05%, the quaternary amine will be present at a concentration of about 0.002 M to about 0.05 M. and the solvent at about 20% to about 60% by volume, the pH being from about 6.0 to about 7.5. The buffer is preferably within the range of about 0.005 to about 0.1 M.

An embodiment of the invention will now be described with reference to the accompanying drawing which shows a block diagram of a drug detection system which is an embodiment of the invention.

The three primary chromatographic columns of the system are contained in a temperature-controlled housing 11 which is maintained at a constant temperature, generally within the range of about 40-45 °C. The three columns are an anion exchange column 12, which contains a packing of styrene-divinylbenzene copolymer with tetramethylammonium functional groups. The average particle size is 11 microns, and the column 10 mm in length and 3.2 mm internal diameter. It is expected that similar columns with lengths up to 30 mm and internal diameter up to 4.6 mm will yield similar results. Downstream of the anion exchange column 12 is the reversed phase column, which is packed with an octyl-bonded silica which has a carbon loading of 9%, an average particle size of about 5 microns and an average pore size of about 120 Angstroms. The column is 20 mm in length and 3.2 mm internal diameter. It is expected that similar results will be obtained by varying the length and diameter within 50%.

The furthest column downstream is the cation exchange column 14, packed with underivatized silica having an average particle size of about 5 microns, an average pore diameter of 50-80 Angstroms, and a surface area exceeding 350 m^2/g, The column is 150 mm in length and 4.0 mm in internal diameter, both variable within 20%.

Upstream of these columns is the precolumn 15, of length 15 mm and internal diameter 2.1 mm, packed with a hydrophobic styrene-divinylbenzene packing, with an average particle size ranging from about 12 to about 20 microns.

The system contains a series of liquid reservoirs 18 which supply the various solvents and carrier liquids to the system. Three of the reservoirs 19, 20, 21 feed a common pump 22, while the fourth reservoir 23 feeds a separate pump 24. The separation of feeds between two pumps permits the flow of two different liquid solutions into different portions of the system at the same time. A sample selection valve 25 provides for variable flow selection among the transfer lines leading from the three reservoirs 19, 20, 21 to the pump 22, so that solutions from the three reservoirs can be combined in variable proportions into a single stream. This valve may be incorporated into the pump 22 itself, such as for example a low-pressure ternary gradient pump. The second pump 24 will generally be an isocratic pump.

In the prototype system, the first liquid reservoir 19 contains the application or sample purging buffer. The second and third liquid reservoirs 20, 21 contain liquids which include the components of the carrier solution distributed between them such that, when these liquids are combined in certain proportions, carrier solutions of the desired strengths are obtained. The fourth liquid reservoir 23 contains a fully constituted carrier liquid of a specified strength which can be pumped through the isocratic pump 24 at the same time that a solution drawn from one or more of the first three reservoirs 19, 20, 21 is pumped through the gradient pump 22.

Test samples to be analyzed enter the system through an automated sampling device 28, which draws precisely measured aliquots (0.5 mL) of each test sample and injects them into the flowing liquid stream emerging from the pump 22 at preselected intervals. Conventional equipment designed for serial sample injection is commercially available and may be used.

The arrangement of liquid flows to the various columns, and the connection and disconnection between the columns is achieved by an 8-way valve 30 and two 4-way valves 31, 32. These are conventional pieces

of equipment commercially available. Each is shown in one of two positions, the other achieved by rotating in either direction through an arc equal to the distance between adjacent ports. The 8-way valve 1 is arranged to pass fluids through the precolumn 15 in either direction, and to direct the column effluent either to waste 33 or to an input port on the 4-way valve 31 immediately downstream. The latter is likewise arranged to receive fluid streams from the two input lines driven respectively by the two pumps 22, 24, and to direct one of these to the analytical column housing 11 and the other to the second 4-way valve 32 which is interposed between the anion exchange column 12 and the reversed phase column 13. The second 4-way valve 32 in turn receives fluid flow from the anion exchange column 12 and the first 4-way valve 31, and directs one of these to the reversed phase column 13 and the other to waste 34.

The stream emerging from the silica column 14 contains the drugs fully separated and ready for detection. The stream passes through a scanning UV detector 35. This unit consists of conventional instrumentation which detects the peaks as they emerge using standard chromatographic detection methods, and further performs a UV absorptivity scan of each peak preferably at multiple points on the peak, such as the midpoints of the leading and trailing sides as well as the apex of the peak itself. Fluids which have passed through the detector are then passed to waste 36.

The information obtained in the detector 35 is monitored and processed by a computer/monitor unit 37. This unit contains a memory library of retention times and UV absorptivity scans for known drugs, and compares the data received from the detector 35 with the library information as a means of establishing the identity of each drug as it passes through the detector 35. The computer/monitor 37 further integrates the peaks to provide information on the relative amounts of the drugs present in the sample. Thus, for each emerging drug, the system determines its identity (by UV scan and retention time) and its quantity (by peak integration). This information is then transmitted to a printer 38, which provides a full printed analysis of UV-absorbing drugs which have reached the detector.

At the center of the system, coordinating the entire sequence of operations is a system controller 39 which controls the sample selection valve 25, and the computer/monitor 37. The controller 39, computer/monitor 37, printer 38 and detector 35 are conventional equipment commercially available and used in the industry for the same or similar functions.

The following is a sequence of events used for drug analysis of serum or urine on the above-described prototype. In this description:

column 1 is the PRP-1 pre-column (element 15 in the drawing)
column 2 is the AMINEX column (element 12)
column 3 is the reversed phase column (element 13)
column 4 is the silica column (element 14)
solvent A is 0.1% borate buffer, pH 8.0
solvent B is a mixture of:
5 mL 1 M KH_2PO_4
150 µL dimethyloctylamine
275 mg tetramethylammonium chloride
645 mL water
pH adjusted to 6.75 ± 0.02 with H_3PO_4 or KOH
solvent C is HPLC grade acetonitrile
valve 1 is the 8-way valve (element 30)
valve 2 is the first 4-way valve (element 31)
valve 3 is the second 4-way valve (element 32)

Duration	Flow Conditions	Event Description
Step 1 -- 0.5 minute	Column 1 rinsed in 100% C at 4.0 mL/min. Columns 2, 3 and 4 in 65% B, 35% C at 1.0 mL/min. Sample pickup in progress.	Column 1 reactivated to prepare for new sample.
Step 2 -- 0.5 minute	Column 1 rinsed in 100% A at 4.0 mL/min. Columns 2, 3 and 4 in 65% B, 35% C at 1.0 mL/min. Sample pickup in progress.	Column 1 rinsed with buffer to prepare for new sample.
Step 3 -- 0.1 minute	Sample pickup completed. Otherwise same conditions as step 2.	Sample transferred to column 1.
Step 4 -- 0.5 minute	Conditions identical to Step 3.	Column 1 rinsed in forward direction.
Step 5 -- 1.5 minutes	Valve 1 reversed. Conditions otherwise identical to step 3.	Column 1 rinsed in reverse direction.
Step 6 -- 0.2 minute	Valve 2 switched to connect all four columns. 60% B, 40% C at 1.0 mL/min passed through.	High strength mobile phase used to dislodge drugs from column 1.
Step 7 -- 0.6 minute	70% B, 30% C at 1.0 mL/min.	Low strength mobile phase used to continue transfer of drugs, concentrating drugs at heads of analytical columns.
Step 8 -- 0.1 minute	65% B, 35% C at 1.0 mL/min.	Fastest drugs have moved from column 2 to column 3; slowest drugs moving from column 1 to column 2.
Step 9 -- 0.5 minute	Valve 2 switched to disconnect columns 1 and 2. 30% B, 70% C passed through column 1 at 1 mL/min; 65% B, 35% C passed through columns 2, 3 and 4 at 1.0 mL/min.	Remaining drugs moving through columns 2 and 3 to column 4. Rinse of column 1 begins.

Step 10 -- 5.2 minutes	Valves 2 and 3 switched to connect columns 1 and 2 and disconnect columns 3 and 4. 1 and 2 receive 30% B, 70% C at 1.0 mL/min; 3 and 4 receives 65% B, 35% C at 1.0 mL/min.	All drugs have passed through columns 1 and 2, which are now being rinsed.
Step 11 -- 5.2 minutes	65% B, 35% C passed through columns 1 and 2 at 1.0 mL/min; columns 3 and 4 flow continued as in step 10.	Columns 1 and 2 being reequilibrated in mobile phase.
Step 12 -- .05 minute	All flow rates lowered to 0.1 mL/min.	End of run.

Note: Data analysis and printing of the report for each sample occurs during steps 1 through 7 of the subsequent sample.

The following is a representative list of drugs for which a sample of serum or urine may be analyzed by use of the scheme described above. This list is merely illustrative and is not intended to be comprehensive.

DETECTABLE DRUGS

Drug (in alphabetical order)	Column(s) Where Retained
alprazolam	reversed phase
amitriptyline	reversed phase, silica
amoxapine	reversed phase, silica
amphetamine	silica
benzoylecgonine	silica
butalbital	anion exchange
chlordiazepoxide	reversed phase
chlorpheniramine	silica
cimetidine	silica
cocaine	silica
codeine	silica
desalkylflurazepam	reversed phase
desipramine	reversed phase, silica
diazepam	reversed phase
diphenhydramine	reversed phase, silica
doxepin	reversed phase, silica
ephedrine	silica
ethclorvynol	reversed phase
glutethimide	anion exchange
imipramine	reversed phase, silica
lidocaine	silica
lorazepam	reversed phase
loxapine	reversed phase, silica
maprotiline	reversed phase, silica
meperidine	silica
methadone	silica
methamphetamine	silica
methaqualone	reversed phase
morphine	silica
oxazepam	anion exchange, reversed phase
pentazocine	silica
phencyclidine	silica
phenobarbital	anion exchange
phentermine	silica
phenylpropanolamine	silica
phenytoin	anion exchange, reversed phase
propoxyphene	silica
pyrilamine	silica
quinidine	reversed phase, silica
secobarbital	anion exchange
thioridazine	reversed phase, silica
tripelennamine	silica

Claims

1. A system for analyzing a biological sample for the presence of drugs in the form of anions, bases and neutral compounds, said system comprising:
   (a) means for purging said sample of inorganic salts and proteins;
   (b) a chromatographic column combination comprising:
      (i) an anion exchange medium;
      (ii) a reversed phase medium;

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DETECTABLE DRUGS

Drug (in alphabetical order)	Column(s) Where Retained
alprazolam	reversed phase
amitriptyline	reversed phase, silica
amoxapine	reversed phase, silica
amphetamine	silica
benzoylecgonine	silica
butalbital	anion exchange
chlordiazepoxide	reversed phase
chlorpheniramine	silica
cimetidine	silica
cocaine	silica
codeine	silica
desalkylflurazepam	reversed phase
desipramine	reversed phase, silica
diazepam	reversed phase
diphenhydramine	reversed phase, silica
doxepin	reversed phase, silica
ephedrine	silica
ethclorvynol	reversed phase
glutethimide	anion exchange
imipramine	reversed phase, silica
lidocaine	silica
lorazepam	reversed phase
loxapine	reversed phase, silica
maprotiline	reversed phase, silica
meperidine	silica
methadone	silica
methamphetamine	silica
methaqualone	reversed phase
morphine	silica
oxazepam	anion exchange, reversed phase
pentazocine	silica
phencyclidine	silica
phenobarbital	anion exchange
phentermine	silica
phenylpropanolamine	silica
phenytoin	anion exchange, reversed phase
propoxyphene	silica
pyrilamine	silica
quinidine	reversed phase, silica
secobarbital	anion exchange
thioridazine	reversed phase, silica
tripelennamine	silica

Claims

1. A system for analyzing a biological sample for the presence of drugs in the form of anions, bases and neutral compounds, said system comprising:
   (a) means for purging said sample of inorganic salts and proteins;
   (b) a chromatographic column combination comprising:
      (i) an anion exchange medium;
      (ii) a reversed phase medium;

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(iii) a cation exchange medium;

(c) a carrier liquid; and

(d) means for identifying said drugs emerging from said chromatographic column combination;

said carrier liquid selected, and said chromatographic column combination arranged, to promote the selective retention of hydrophobic anions and neutral compounds and the chromatographic separation of weak acids on said anion exchange medium, the chromatographic separation of weak bases, hydrophobic bases and neutral compounds on said reversed phase medium, and the chromatographic separation of bases on said cation exchange medium.

2. A system in accordance with claim 1 in which said purging means is comprised of a hydrophobic styrene-divinylbenzene copoplymer resin and an aqueous buffer solution at a pH of about 7.5 to about 9.0.

3. A system in accordance with claim 1 in which said anion exchange medium is a polymeric resin with quaternary ammonium functional sites.

4. A system in accordance with claim 1 in which said reversed phase medium is a derivatized silica.

5. A system in accordance with claim 1 in which said cation exchange medium is comprised of underivatized silica.

6. A system in accordance with claim 1 in which:

said purging means is comprised of a hydrophobic styrene-divinylbenzene copolymer resin,

said anion exchange medium is a styrene-divinylbenzene resin with tetramethylammonium functional sites in a packed column of about 10 mm to about 30 mm in length;

said reversed phase medium is octyl-bonded silica with a carbon content of about 8% to about 10% by weight in a packed column of about 10 mm to about 50 mm in length; and

said cation exchange medium is underivatized silica in a packed column of about 100 mm to about 250 mm in length.

7. A system in accordance with claim 1 in which said carrier liquid contains acetonitrile at about 10% to about 50% by volume, dimethyloctylamine at about 30 to about 300 microliters per liter, and a member selected from the group consisting of tetraalkylammonium hydroxides and halides at about 0.1 to 2.0 g per liter.

8. A system in accordance with claim 1 further comprising:

a first stock liquid containing at least about 75% acetonitrile by volume;

a second stock liquid comprising an aqueous buffer solution having dissolved therein from about 0.001% to about 0.05% by volume of alkylamine with a carbon chain of at least 6 carbon atoms, from about 0.002 M to about 0.05 M of a quaternary amine, and from about 20% to about 60% by weight of a water-soluble organic solvent, at a pH of from about 6.0 to about 7.5; and

variable mixing means for combining said first and second stock solutions to form said carrier liquid, in a plurality of proportions to vary the concentration of acetonitrile in said carrier liquid.

9. A system in accordance with claim 8 further comprising control means for controlling said variable mixing means and for directing said carrier liquid to preselected portions of said chromatographic column combination in a preselected sequence, to condition portions of said chromatographic column combination while said drugs are passing through remaining portions thereof.

10. A system in accordance with claim 1 in which said identifying means comprises:

(a) means for automatically scanning each said drug emerging from said chromatographic column combination to produce an absorption spectrum characteristic of each said drug; a library of presupplied spectra of known drugs; and means for automatically comparing spectra produced by said scanning means with said presupplied spectra;

(b) means for automatically detecting retention times of said drugs as they emerge from said chromatographic column combination; a library of predetermined retention times for known drugs; and means for automatically comparing retention times so detected with said predetermined retention times; and

(c) peak integrating means for determining the relative amounts of said drugs in said sample as said drugs emerge from said chromatographic column combination.

11. A method for analyzing a biological sample for the presence of drugs in the form of anions, bases and neutral compounds, said method comprising:
(a) removing from said sample inorganic salts and proteins contained therein;
(b) passing the components of said sample retained in step (a) through the following media in sequence:
(i) an anion exchange medium under conditions permitting selective retention of hydrophobic anions and neutral compounds, with substantially no retention of bases;
(ii) a reversed phase medium under conditions permitting chromatographic separation of weak bases, hydrophobic bases and neutral compounds, with substantially no retention of hydrophilic bases and anions; and
(iii) a cation exchange medium under conditions permitting chromatographic separation of bases, with substantially no retention of anions; and
(c) detecting the drugs emerging from said cation exchange medium.

12. A method according to claim 11 comprising:
as step (a) impregnating an underivatized styrene-divinylbenzene copolymer resin with said sample and purging said resin with an aqueous solution of a borate buffer;
as step (b) passing the components of said sample retained in step (a) through the following media in sequence:
(i) a column of styrene-divinylbenzene resin with quaternary ammonium functional sites, said column ranging from about 10 mm to about 30 mm in length;
(ii) a column of octyl-bonded silica with a carbon content of about 6% to about 12% by weight, said column ranging from about 10 mm to about 50 mm in length; and
(iii) an underivatized silica column ranging from about 100 mm to about 250 mm in length;
by way of a carrier liquid containing acetonitrile at about 10% to about 50% by volume, dimethyloctylamine at about 30 to about 300 µL per liter, and a tetraalkylammonium ion at about 0.1 to about 2.0 g per liter, and
(c) detecting said drugs as they emerge from said underivatized silica column.

13. A method according to claim 11 wherein the sample is carried by a carrier solution made up from two stock solutions, the first being primarily acetonitrile in solution, the second containing a long chain alkylamine, a quaternary amine, a buffer and solvent.

Patentansprüche

1. System zum Analysieren einer biologischen Probe auf die Gegenwart von Arzneimitteln bzw. Drogen in Form von Anionen, Basen und neutralen Verbindungen, wobei das genannte System umfaßt:
(a) Mittel zum Reinigen der genannten Probe von anorganischen Salzen und Proteinen;
(b) eine Chromatographiesäulenkombination, umfassend:
(i) ein Anionenaustauschmedium;
(ii) ein Umkehrphasenmedium;
(iii) ein Kationenaustauschmedium;
(c) eine Trägerflüssigkeit; und
(d) Mittel zum Identifizieren der genannten Arzneimittel bzw. Drogen, die aus der genannten Chromatographiesäulenkombination herauskommen;
wobei die genannte Trägerflüssigkeit so ausgewählt und die genannte Chromatographiesäulenkombination so angeordnet ist, daß die selektive Retention hydrophober Anionen und neutraler Verbindungen und die chromatographische Trennung schwacher Säuren auf dem genannten Anionenaustauschmedium, die chromatographische Trennung schwacher Basen, hydrophober Basen und neutraler Verbindungen auf dem genannten Umkehrphasenmedium und die chromatographische Trennung von Basen auf dem genannten Kationenaustauschmedium gefördert werden.

2. System nach Anspruch 1, bei dem das genannte Reinigungsmittel aus einem hydrophoben Styrol-Divinylbenzol-Copolymerharz und einer wässerigen Pufferlösung mit einem pH-Wert von etwa 7,5 bis etwa 9,0 besteht.

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3. System nach Anspruch 1, bei dem das genannte Anionenaustauschmedium ein Polymerharz mit funktionellen quaternären Ammoniumstellen ist.

4. System nach Anspruch 1, bei dem das genannte Umkehrphasenmedium derivatisierte Silika ist.

5. System nach Anspruch 1, bei dem das genannte Kationenaustauschmedium aus underivatisierter Silika besteht.

6. System nach Anspruch 1, bei dem
das genannte Reinigungsmittel aus einem hydrophoben Styrol-Divinylbenzol-Copolymerharz besteht,
das genannte Anionenaustauschmedium ein Styrol-Divinylbenzolharz mit funktionellen Tetramethylammoniumstellen in einer bepackten Säule mit etwa 10 mm bis etwa 30 mm Länge ist;
das genannte Umkehrphasenmedium octylgebundene Silika mit einem Kohlenstoffgehalt von etwa 8 bis etwa 10 Gew.-% in einer bepackten Säule mit etwa 10 mm bis etwa 50 mm Länge ist; und
das genannte Kationenaustauschmedium underivatisierte Silika in einer bepackten Säule mit etwa 100 mm bis etwa 250 mm Länge ist.

7. System nach Anspruch 1, bei dem die genannte Trägerflüssigkeit Acetonitril in einer Menge von etwa 10 Vol-% bis etwa 50 Vol-%, Dimethyloctylamin in einer Menge von etwa 30 bis etwa 300 Mikroliter pro Liter und ein Mitglied, das aus der Gruppe ausgewählt ist, die aus Tetraalkylammoniumhydroxiden und Haliden besteht, in einer Menge von etwa 0,1 bis 2,0 g pro Liter enthält.

8. System nach Anspruch 1, das weiters umfaßt:
eine erste Stammflüssigkeit, die zumindest etwa 75 Vol-% Acetonitril enthält;
eine zweite Stammflüssigkeit, die eine wässerige Pufferlösung umfaßt, die darin von etwa 0,001 Vol-% bis etwa 0,05 Vol-% Alkylamin mit einer Kohlenstoffkette aus zumindest 6 C-Atomen, von etwa 0,002 M bis etwa 0,05 M eines quaternären Amins, und von etwa 20 Gew.-% bis etwa 60 Gew.-% eines wasserlöslichen organischen Lösungsmittels aufgelöst aufweist, mit einem pH-Wert von etwa 6,0 bis etwa 7,5; und
verstellbare Mischeinrichtungen zum Kombinieren der genannten ersten und zweiten Stammlösungen, um die genannte Trägerflüssigkeit zu bilden, in einer Vielzahl von Verhältnissen, um die Konzentration von Acetonitril in der genannten Trägerflüssigkeit zu variieren.

9. System nach Anspruch 8, das weiters Steuermittel zum Steuern der genannten verstellbaren Mischeinrichtung und zum Lenken der genannten Trägerflüssigkeit zu vorher ausgewählten Abschnitten der genannten Chromatographiesäulenkombination in einer vorher ausgewählten Abfolge umfaßt, um Abschnitte der genannten Chromatographiesäulenkombination zu konditionieren, während die genannten Arzneimittel bzw. Drogen durch verbleibende Abschnitte davon geschickt werden.

10. System nach Anspruch 1, bei dem das genannte Identifizierungsmittel umfaßt:
(a) Mittel zum automatischen Abtasten eines jeden genannten Arzneimittels bzw. Droge, das aus der genannten Chromatographiesäulenkombination herauskommt, um ein Absorptionsspektrum zu erhalten, das für jedes genannte Arzneimittel charakteristisch ist; eine Sammlung vorher zur Verfügung gestellter Spektren bekannter Arzneimittel bzw. Drogen; und Mittel zum automatischen Vergleichen der vom genannten Abtastmittel erzeugten Spektren mit den genannten vorher zur Verfügung gestellten Spektren;
(b) Mittel zum automatischen Ermitteln der Retentionszeiten der genannten Arzneimittel, wenn sie aus der genannten Chromatographiesäulenkombination herauskommen; eine Sammlung vorher bestimmter Retentionszeiten für bekannte Arzneimittel; und Mittel zum automatischen Vergleichen der so ermittelten Retentionszeiten mit den genannten vorher bestimmten Retentionszeiten; und
(c) Peakintegrationsmittel zum Bestimmen der relativen Mengen der genannten Arzneimittel in der genannten Probe, wenn die genannten Arzneimittel aus der genannten Chromatographiesäulenkombination kommen.

11. Verfahren zum Analysieren einer biologischen Probe auf die Gegenwart von Arzneimitteln bzw. Drogen in der Form von Anionen, Basen und neutralen Verbindungen, wobei das genannte Verfahren umfaßt:
(a) das Entfernen von darin enthaltenen anorganischen Salzen und Proteinen aus der genannten Probe;

(b) das Hindurchschicken der Bestandteile der genannten in Schritt (a) zurückgehaltenen Probe der Reihe nach durch die folgenden Medien:

(i) ein Anionenaustauschmedium unter Bedingungen, die die selektive Retention hydrophober Anionen und neutral er Verbindungen im wesentlichen ohne Retention von Basen zulassen;

(ii) ein Umkehrphasenmedium unter Bedingungen, die die chromatographische Trennung schwacher Basen, hydrophober Basen und neutral er Verbindungen im wesentlichen ohne Retention hydrophiler Basen und Anionen zulassen; und

(iii) ein Kationenaustauschmedium unter Bedingungen, die die chromatographische Trennung von Basen im wesentlichen ohne Retention von Anionen zuläßt; und

(c) das Nachweisen von Arzneimitteln bzw. Drogen, die aus dem genannten Kationenaustauschmedium herauskommen.

12. Verfahren nach Anspruch 11, umfassend:

als Schritt (a) das Tränken eines underivatisierten Styrol-Divinylbenzol-Copolymerharzes mit der genannten Probe und das Reinigen des genannten Harzes mit einer wässerigen Lösung eines Boratpuffers;

als Schritt (b) das Hindurchschicken der Bestandteile der genannten in Schritt (a) zurückgehaltenen Probe der Reihe nach durch folgende Medien:

(i) eine Säule aus Styrol-Divinylbenzol-Harz mit funktionellen quaternären Ammoniumstellen, wobei die genannte Säule eine Länge im Bereich von etwa 10 mm bis etwa 30 mm aufweist;

(ii) eine Säule aus Octyl-gebundener Silika mit einem Kohlenstoffgehalt von etwa 6 Gew.-% bis etwa 12 Gew.-%, wobei die genannte Säule eine Länge im Bereich von etwa 10 mm bis etwa 50 mm aufweist; und

(iii) eine Säule aus underivatisierter Silika mit einer Länge im Bereich von etwa 100 mm bis etwa 250 mm;

mittels einer Trägerflüssigkeit, die Acetonitril in einer Menge von etwa 10 Vol-% bis etwa 50 Vol-%, Dimethyloctylamin in einer Menge von etwa 30 bis etwa 300 µL pro Liter, und ein Tetraalkylammoniumion mit etwa 0,1 bis etwa 2,0 g pro Liter enthält, und

(c) das Nachweisen der genannten Arzneimittel bzw. Drogen, wenn sie aus der genannten Säule aus underivatisierter Silika herauskommen.

13. Verfahren nach Anspruch 11, worin die Probe von einer Trägerlösung getragen wird, die aus zwei Stammlösungen gebildet ist, wobei die erste hauptsächlich Acetonitril in Lösung ist, die zweite ein langkettiges Alkylamin, ein quaternäres Amin, einen Puffer und Lösungsmittel enthält.

Revendications

1. Système destiné à analyser un échantillon biologique pour déterminer la présence de médicaments sous forme d'anions, de bases et de composés neutres, ce système comprenant :

(a) un moyen pour purger, de l'échantillon, les sels minéraux et les protéines ;

(b) une combinaison de colonnes chromatographiques comprenant :

(i) un milieu d'échange d'anions ;

(ii) un milieu à phase inversée ;

(iii) un milieu d'échange de cations ;

(c) un liquide porteur ; et

(d) un moyen pour identifier les médicaments sortant de la combinaison de colonnes chromatographiques ; le liquide support étant sélectionné, et la combinaison de colonnes chromatographique étant disposée de façon à favoriser la rétention sélective des anions hydrophobes et des composés neutres ainsi que la séparation chromatographique des acides faibles sur le milieu d'échange d'anions, la séparation chromatographique des bases faibles, des bases hydrophobes et des composés neutres sur le milieu à phase inversée, et la séparation chromatographique des bases sur le milieu d'échange de cations.

2. Système selon la revendication 1, dans lequel le moyen de purge est constitué d'une résine hydrophobe à base d'un copolymère styrène-divinylbenzène, et d'une solution tampon aqueuse à pH compris entre environ 7,5 et environ 9,0.

3. Système selon la revendication 1, dans lequel le milieu d'échange d'anions est une résine polymère comportant des sites fonctionnels ammonium quaternaires.

4. Système selon la revendication 1, dans lequel le milieu à phase inversée est constitué d'une silice transformée.

5. Système selon la revendication 1, dans lequel le milieu d'échange de cations est constitué d'une silice non transformée.

6. Système selon la revendication 1, dans lequel :

le milieu de purge est constitué d'une résine hydrophobe à base d'un copolymère styrène-divinylbenzène ;

le milieu d'échange de cations est une résine styrène-divinylbenzène comportant des sites fonctionnels tétraméthylammonium dans une colonne garnie ayant une longueur d'environ 10 mm à environ 30 mm ;

le milieu à phase inversée est constitué d'une silice à liaison octyle ayant une teneur en carbone d'environ 8 % à 10 % en poids, dans une colonne garnie ayant une longueur d'environ 10 mm à environ 50 mm ; et

le milieu d'échange de cations est constitué d'une silice non transformée, dans une colonne garnie ayant une longueur d'environ 100 mm à environ 250 mm.

7. Système selon la revendication 1, dans lequel le liquide porteur contient de l'acétonitrile en une quantité d'environ 10 % à environ 50 % en volume, de la diméthyloctylamine à raison d'environ 30 microlitres à environ 300 microlitres par litre, et un composé choisi parmi l'ensemble comprenant les hydroxydes et halogénures de tétraalkylammonium en une quantité d'environ 0,1 à 2,0 g par litre.

8. Système selon la revendication 1, qui comprend en outre :

une première liqueur mère contenant au moins environ 75 % en volume d'acétonitrile ;

une deuxième liqueur mère, comprenant une solution tampon aqueuse dans laquelle sont dissous d'environ 0, 001 à environ 0,05 % en volume d'une alkylamine ayant une chaîne carbonée constituée d'au moins 6 atomes de carbone, une amine quaternaire en une quantité d'environ 0,002 M à environ 0,05 M, et une quantité d'un solvant organique soluble dans l'eau comprise entre environ 20 % et environ 60 % en poids, à un pH compris entre environ 6,0 et environ 7,5 ; et

des moyens de mélange variables, permettant de combiner la première et la deuxième liqueurs mères pour former le liquide porteur, selon de nombreuses proportions destinées à faire varier la concentration de l'acétonitrile dans le liquide porteur.

9. Système selon la revendication 8, comprenant en outre un moyen de régulation destiné à réguler le moyen de mélange variable, et à envoyer le liquide porteur dans des parties prédéfinies de la combinaison de colonnes chromatographiques, selon une séquence pré-sélectionnée, pour conditionner des parties de la combinaison de colonnes chromatographiques quand les médicaments traversent les autres parties de cette dernière.

10. Système selon la revendication 1, dans lequel le moyen d'identification comprend :

(a) un moyen pour analyser automatiquement chaque médicament sortant de la combinaison de colonnes chromatographiques, pour obtenir un spectre d'absorption caractéristique de chacun des médicaments ; une bibliothèque de spectres de médicaments connus ; et un moyen pour comparer automatiquement les spectres produits par le moyen d'analyse, et les spectres pré-définis ;

(b) un moyen pour détecter automatiquement les temps de rétention des médicaments quand ils sortent de la combinaison de colonnes chromatographiques ; une bibliothèque de temps de rétention pré-définis de médicaments connus ; et un moyen pour comparer automatiquement les temps de rétention ainsi détectés aux temps de rétention pré-déterminés ; et

(c) un moyen d'intégration des pics pour déterminer les quantités relatives des médicaments dans l'échantillon quand les médicaments sortent de la combinaison de colonnes chromatographiques.

11. Procédé pour analyser un échantillon biologique pour y déterminer la présence de médicaments sous forme d'anions, de bases et de composés neutres, ce procédé consistant :

(a) à extraire de l'échantillon les sels minéraux et les protéines qui y sont contenus ;

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(b) à faire passer les composants de l'échantillon, retenus dans l'étape (a) successivement par les milieux suivants :

(i) un milieu d'échange d'anions, dans des conditions permettant la rétention sélective des anions hydrophobes et des composés neutres, la rétention des bases étant négligeable ;

(ii) un milieu à phase inversée, dans des conditions permettant de séparer par chromatographie des bases faibles, des bases hydrophobes et des composés neutres, la rétention des bases hydrophiles et des anions étant négligeable ; et

(iii) un milieu d'échange de cations, dans des conditions permettant une séparation chromatographique des bases, la rétention des anions étant négligeable ; et

(c) à détecter les médicaments sortant du milieu d'échange de cations.

12. Procédé selon la revendication 11, qui consiste :

dans l'étape (a), à imprégner de l'échantillon une résine non transformée constituée d'un copolymère styrènedivinylbenzène, et à purger la résine avec une solution aqueuse d'un tampon borate ;

dans l'étape (b), à faire passer les composants de l'échantillon, retenus dans l'étape (a) successivement dans les milieux suivants :

(i) une colonne d'une résine de styrènedivinylbenzène comportant des sites fonctionnels ammonium quaternaire, la longueur de la colonne étant comprise entre environ 10 mm et environ 30 mm ;

(ii) une colonne de silice à liaison octyle ayant une teneur en carbone d'environ 6 % à environ 12 % en poids, la longueur de la colonne étant comprise entre environ 10 mm et environ 50 mm ; et

(iii) une colonne de silice non transformée ayant une longueur comprise entre environ 100 mm et environ 250 mm ; à l'aide d'un liquide support contenant de l'acétonitrile en une quantité d'environ 10 % à environ 50 % en volume, de la diméthyloctylamine à raison d'environ 30 à environ 300 microlitres par litre, et un ion tétraalkylammonium en une quantité d'environ 0,1 à environ 2,0 g par litre, et

(c) à détecter les médicaments quand ils sortent de la colonne de silice non transformée.

13. Procédé selon la revendication 11, dans lequel l'échantillon est porté par une solution support constituée de deux solutions mères, la première étant constituée essentiellement d'acétonitrile en solution, la deuxième contenant une alkylamine à longue chaîne, une amine quaternaire, un tampon et un solvant.