Form PTO-1595 (Rev. 06-12)
OMB No. 0651-0027 (exp. 04/30/2015)

12/29/2015

103674181

U.S. DEPARTMENT OF COMMERCE
United States Patent and Trademark Office

RECC

12/29/15

To the Director of the U.S. Patent and Trademark Office: Please record the attached documents or the new address(es) below.

1. Name of conveying party(ies)

William E. Brewer

Additional name(s) of conveying party(ies) attached? ☐ Yes ☐ No

2. Name and address of receiving party(ies)

Name: Business Development Corporation of South Carolina
Internal Address: P.O. Box 21823
Columbia, South Carolina 29221
Street Address: 111 Executive Center Drive
Enoree Bldg., Ste. 225
City: Columbia
State: South Carolina
Country: USA Zip: 29210

Additional name(s) & address(es) attached? ☐ Yes ☒ No

3. Nature of conveyance/Execution Date(s):

Execution Date(s) December 18, 2015

☐ Assignment ☐ Merger
☒ Security Agreement ☐ Change of Name
☐ Joint Research Agreement
☐ Government Interest Assignment
☐ Executive Order 9424, Confirmatory License
☐ Other

4. Application or patent number(s):

☒ This document serves as an Oath/Declaration (37 CFR 1.63).

A. Patent Application No.(s)

14/379,227

B. Patent No.(s)

6,566,145 8,715,593

Additional numbers attached? ☐ Yes ☒ No

5. Name and address to whom correspondence concerning document should be mailed:

Name: Dina G. Boorda, Esquire
Internal Address: P.O. Box 21215
Columbia, South Carolina 29221
Street Address: 111 Executive Center Drive
Enoree Bldg., Ste. 231
City: Columbia
State: South Carolina Zip: 29210
Phone Number: (803)772-0965
Docket Number:
Email Address: dinaboorda@callisontighe.com

6. Total number of applications and patents involved: three (3)

7. Total fee (37 CFR 1.21(h) & 3.41) $120.00

☐ Authorized to be charged to deposit account
☒ Enclosed
☐ None required (government interest not affecting title)

8. Payment Information

Deposit Account Number 12/29/2015 KNGUYEN1 00000018 14379227
01 FC:8021 120.00 OP

Authorized User Name

9. Signature:

Signature

December 18, 2015
Date

William E. Brewer
Name of Person Signing

Total number of pages including cover sheet, attachments, and documents: 49

Documents to be recorded (including cover sheet) should be faxed to (571) 273-0140, or mailed to:
Mail Stop Assignment Recordation Services, Director of the USPTO, P.O.Box 1450, Alexandria, V.A. 22313-1450

Business Development Corporation of South Carolina

TERM FINANCING IN
SBA 7(A) LOAN

SECURITY AGREEMENT

THIS SECURITY AGREEMENT ("**Agreement**") is executed and delivered this 18th day of December, 2015, by **DPX Technologies, LLC** and **DPX Labs, LLC,** and **William E. Brewer** (hereinafter collectively referred to as "**Debtor**"), in favor of **Business Development Corporation of South Carolina,** its successors and assigns ("**Secured Party**").

WITNESSETH:

WHEREAS, Debtors, DPX Technologies, LLC and DPX Labs, LLC are indebted to Secured Party pursuant to their promissory note dated of even date herewith, in the principal amount of One Million Four Hundred Thirty-Five Thousand and No/100s---------- ($1,435,000.00) Dollars together with any and all extensions, renewals, or modifications thereof (the "**BDC Note**") and pursuant to the terms and conditions set forth in that certain loan agreement of even date therewith (the "**Loan Agreement**") entered into by and between Debtor(s), Secured Party;

WHEREAS, William E. Brewer, Guarantor is indebted to Secured Party pursuant to his unlimited Unconditional Guarantee, dated of even date herewith; and

WHEREAS, U.S. Small Business Administration ("**SBA**") has agreed to provide financing to Debtor pursuant to the terms and conditions of that certain Authorization dated September 18, 2015, with any amendments thereto, (the "**SBA Authorization**") in the amount of One Million Four Hundred Thirty-Five Thousand and No/100s---------- Dollars ($1,435,000.00) (the "**SBA Loan**").

WHEREAS, the Debtor desires to secure its obligations under the Lender's Note and by granting to the Secured Party a security interest in the property described below;

NOW, THEREFORE, in consideration of the foregoing and to induce Lender to make the SBA Loan to Debtor(s), and for other good and valuable consideration, the receipt and sufficiency of which is hereby acknowledged, the parties agree as follows:

1. **Grant of Security Interest**. The Debtor hereby grants to the Secured Party, to secure the payment and performance in full of all of the Obligations (as defined below), a security interest in and pledges and assigns to the Secured Party the following properties, assets, and rights of the Debtor (all of the same being in this Agreement called the "**Collateral**"):

(a) All of Debtors' now owned and hereafter acquired furniture, fixtures, machinery and equipment, and all replacements and substitutions therefor and thereof, and all accessions thereto (the "FF&E");

(b) All of Debtors' now owned and hereafter acquired inventory, and all products, replacements, and substitutions therefore and thereof, and all accessions thereto (the "Inventory");

(c) All of Debtor's now owned and hereafter acquired general intangibles, including without limitation, all licenses, permits, things in action, contractual rights, goodwill, literary rights, rights to performance, copyrights, trademark, and patents including without limitation, all rights and interests in and to US Patent No. 6,566,145 and 8,715,593 and US Patent Application No. 14/379,227, now existing or hereafter acquired, developed, licensed, or recorded, and all interests in and to License Agreements of Debtors, subject to the conditions set forth therein (the "General Intangibles");

(d) All of Debtor's now owned and hereafter acquired rights to payment for goods sold or leased or for services rendered (the "Accounts");

(e) All of Debtor's now owned and hereafter acquired chattel paper (the "Chattel Paper"); and

(f) All of Debtor's now owned and hereafter acquired instruments, notes, items of payment, negotiable documents, and documents of title (the "Instruments"); together with all cash and noncash proceeds (including insurance proceeds) of the FF&E, Inventory, General Intangibles, Accounts, Chattel Paper, and Instruments (the "Proceeds") (such Equipment, Inventory, General Intangibles, Accounts, Chattel Paper, Instruments, and Proceeds are collectively referred to as the "Collateral").

(g) Also including, but not limited to, the Fixed Asset Listing attached hereto and incorporated herein by reference, wherever located.

2. **Obligations Secured**. The security interest granted herein secure the following obligations (collectively, the "**Obligations**"): (a) the obligations of the Debtor to Secured Party under the Lender's Note; (b) any and all advances or expenditures made by Secured Party pursuant to the terms of this Agreement; (c) attorneys' fees, court costs, and other amounts which may be due under the Lender's Note or this Agreement; (d) any and all other indebtedness of Debtor to Secured Party, now existing or hereafter arising, of whatever class or nature, whether or not now contemplated by the parties, including future advances; and (e) any and all extensions, renewals, and modifications of any of the foregoing.

3. **Authorization to File Financing Statements**. The Debtor hereby irrevocably authorizes the Secured Party at any time and from time to time to file in any filing office in any Uniform Commercial Code jurisdiction any initial financing statements and amendments to this Agreement that: (a) describe the Collateral, and (b) provide any other information required by part 5 of Article 9 of the Uniform Commercial Code for the sufficiency or filing office acceptance of any financing statement or amendment, including (i) whether the Debtor is an organization, the type of organization and any organizational identification number issued to the Debtor and, (ii) in the case of a financing statement filed as a fixture filing or indicating Collateral as as-extracted collateral or timber to be cut, a sufficient description of real property to which the Collateral relates. The Debtor agrees to furnish any such information to the Secured Party promptly upon the Secured Party's request. The Debtor also ratifies its authorization for the Secured Party to have filed in

any Uniform Commercial Code jurisdiction any like initial financing statements or amendments to this Agreement if filed before the date of this Agreement.

4. **Other Actions for Any and All Collateral**. The Debtor further agrees, upon request of the Secured Party and at the Secured Party's option, to take any and all other actions as the Secured Party may determine to be necessary or useful for the attachment, perfection and first priority of, and the ability of the Secured Party to enforce, the Secured Party's security interest in any and all of the Collateral, including, without limitation, (a) executing, delivering and, where appropriate, filing financing statements and amendments relating to this Agreement under the Uniform Commercial Code, to the extent, if any, that the Debtor's signature thereon is required therefor, (b) causing the Secured Party's name to be noted as secured party on any certificate of title for a titled good if such notation is a condition to attachment, perfection or priority of, or ability of the Secured Party to enforce, the Secured Party's security interest in such Collateral, (c) complying with any provision of any statute, regulation or treaty of the United States as to any Collateral if compliance with such provision is a condition to attachment, perfection or priority of, or ability of the Secured Party to enforce, the Secured Party's security interest in such Collateral, (d) obtaining governmental and other third party waivers, consents and approvals in form and substance satisfactory to the Secured Party, including, without limitation, any consent of any licensor, lessor or other person obligated on Collateral, (e) obtaining waivers from mortgagees and landlords in form and substance satisfactory to the Secured Party, and (f) taking all actions under any earlier versions of the Uniform Commercial Code or under any other law, as reasonably determined by the Secured Party to be applicable in any relevant Uniform Commercial Code or other jurisdiction, including any foreign jurisdiction.

5. **Covenants Concerning Debtor's Legal Status**. The Debtor covenants with the Secured Party as follows: (a) without providing at least 30 days prior written notice to the Secured Party, the Debtor will not change its name, its place of business or, if more than one, chief executive office, or its mailing address or organizational identification number if it has one, (b) if the Debtor does not have an organizational identification number and later obtains one, the Debtor will forthwith notify the Secured Party of such organizational identification number, and (c) the Debtor will not change its type of organization, jurisdiction of organization, or other legal structure.

6. **Representations and Warranties Concerning Collateral, Etc**. The Debtor further represents and warrants to the Secured Party the Debtor is the owner of the Collateral, free from any right or claim of any person or any adverse lien, security interest or other encumbrance, except for the security interest created by this Agreement.

7. **Covenants Concerning Collateral, etc**. The Debtor further covenants with the Secured Party as follows:

(a) except for the security interest in this Agreement granted, the Debtor shall be the owner of the Collateral free from any right or claim of any other person or any lien, security interest or other encumbrance, and the Debtor shall defend the same against all claims and demands of all persons at any time claiming the same or any interests tin this Agreement adverse to the Secured Party;

(b) the Debtor shall not pledge, mortgage or create, or suffer to exist any right of any person in or claim by any person to the Collateral, or any security interest, lien or other encumbrance in the Collateral in favor of any person, other than the Secured Party, except as provided and contemplated in the Loan Agreement;

(c) the Debtor will pay promptly when due all taxes, assessments, governmental charges and levies upon the Collateral or incurred in connection with the use or operation of such Collateral or incurred in connection with this Agreement;

(d) the Debtor will not sell or otherwise dispose, or offer to sell or otherwise dispose, of the Collateral;

(e) the Collateral shall be kept at Debtor's business location and the Debtor will not remove the Collateral from such locations, without the written consent of the Secured Party.

8. **Maintenance of Insurance**. The Debtor shall have and maintain at all times with respect to the Collateral insurance satisfactory to the Secured Party against risks customarily insured against with respect to such types of Collateral, with reputable insurers, in amounts at least equal to current market value of the Collateral, all as reasonably determined by the Secured Party, and containing such terms, in such form, and for such periods as may be reasonably satisfactory to the Secured Party. All such insurance shall be payable to the Secured Party as loss payee under a "standard" or "New York" loss payee clause.

9. **Insurance Proceeds**. The proceeds of any casualty insurance in respect of any casualty loss of any of the Collateral shall, subject to the rights, if any, of other parties with an interest having priority in the property covered thereby, (a) so long as no Event of Default has occurred and is continuing and to the extent that the amount of such proceeds is less than $10,000.00, be disbursed to the Debtor for direct application by the Debtor solely to the repair or replacement of the Debtor's property so damaged or destroyed and (b) in all other circumstances, be held by the Secured Party as cash collateral for the Obligations. The Secured Party may, at its sole option, disburse from time to time all or any part of such proceeds so held as cash collateral, upon such terms and conditions as the Secured Party may reasonably prescribe, for direct application by the Debtor solely to the repair or replacement of the Debtor's property so damaged or destroyed, or the Secured Party may apply all or any part of such proceeds to the Obligations.

10. **Continuation of Insurance**. All policies of insurance shall provide for at least 30 days prior written cancellation notice to the Secured Party. In the event of failure by the Debtor to provide and maintain insurance as in this Agreement provided, the Secured Party may, at its option, provide such insurance and charge the amount thereof to the Debtor. The Debtor shall furnish the Secured Party with certificates of insurance and policies evidencing compliance with the foregoing insurance provisions. In the event of failure to provide and maintain insurance as herein provided, the Secured Party may, at its option, provide such insurance and the Debtor hereby promises to pay to the Secured Party on demand the amount of any reasonable disbursements made by the Secured Party for such purpose. Risk of loss or damage shall accrue to the Debtor to the extent of any deficiency in any effective insurance.

11. **Expenses Incurred by Secured Party**. In the Secured Party's discretion, if the Debtor fails to do so, the Secured Party may discharge taxes and other encumbrances at any time levied or placed on any of the Collateral, make repairs under this Agreement and pay any necessary filing fees or insurance premiums. The Debtor agrees to reimburse the Secured Party on demand for all expenditures so made. The Secured Party shall have no obligation to the Debtor to make any such expenditures, nor shall the making of such expenditures be construed as a waiver or cure any Event of Default.

12. **Secured Party's Obligations and Duties**. Anything in this Agreement to the contrary notwithstanding, the Debtor shall remain obligated and liable under each contract or agreement comprised in the Collateral to be observed or performed by the Debtor thereunder. The Secured Party shall not have any obligation or liability under any such contract or agreement by reason of or arising out of this Agreement or the receipt by the Secured Party of any payment relating to any of the Collateral, nor shall the Secured Party be obligated in any manner to perform any of the obligations of the Debtor under or under any such contract or agreement, to make inquiry as to the nature or sufficiency of any payment received by the Secured Party in respect of the Collateral or as to the sufficiency of any performance by any party under any such contract or agreement, to present or file any claim, to take any action to enforce any performance or to collect the payment of any amounts which may have been assigned to the Secured Party or to which the Secured Party may be entitled at any time or times. The Secured Party's sole duty with respect to the custody, safe keeping and physical preservation of the Collateral in its possession, under § 9-207 of the

Uniform Commercial Code or otherwise, shall be to deal with such Collateral in the same manner as the Secured Party deals with similar property for its own account.

13. **Appointment and Powers of Secured Party**. The Debtor hereby irrevocably constitutes and appoints the Secured Party and any officer or agent of the Secured Party, with full power of substitution, as its true and lawful attorneys-in-fact with full irrevocable power and authority in the place and stead of the Debtor or in the Secured Party's own name, for the purpose of carrying out the terms of this Agreement, to take any and all appropriate action and to execute any and all documents and instruments that may be necessary or useful to accomplish the purposes of this Agreement and, without limiting the generality of the foregoing, hereby gives said attorneys the power and right, on behalf of the Debtor, without notice to or assent by the Debtor, to do the following: (a) upon the occurrence and during the continuance of an Event of Default, generally to sell, transfer, pledge, make any agreement with respect to or otherwise dispose of or deal with any of the Collateral in such manner as is consistent with the applicable state Uniform Commercial Code and as fully and completely as though the Secured Party were the absolute owner thereof for all purposes, and to do, at the Debtor's expense, at any time, or from time to time, all acts and things which the Secured Party deems necessary or useful to protect, preserve or realize upon the Collateral and the Secured Party's security interest in this Agreement, to effect the intent of this Agreement, all no less fully and effectively as the Debtor might do, including, without limitation, to the extent that the Debtor's authorization given in section 3 is not sufficient, to file such financing statements with respect to this Agreement, with or without the Debtor's signature, or a photocopy of this Agreement in substitution for a financing statement, as the Secured Party may deem appropriate and to execute in the Debtor's name such financing statements and amendments to this Agreement and continuation statements which may require the Debtor's signature. To the extent permitted by law, the Debtor hereby ratifies all that said attorneys shall lawfully do or cause to be done by virtue of this Agreement. This power of attorney is a power coupled with an interest and is irrevocable. The powers conferred on the Secured Party under this Agreement are solely to protect its interests in the Collateral and shall not impose any duty upon it to exercise any such powers. The Secured Party shall be accountable only for the amounts that it actually receives as a result of the exercise of such powers, and neither it nor any of its officers, directors, employees, or agents shall be responsible to the Debtor for any act or failure to act, except for the Secured Party's own gross negligence or willful misconduct.

14. **Events of Default**. All Obligations shall, at the option of the Secured Party and notwithstanding any time or credit allowed for payment thereof pursuant to any provision, understanding, or agreement between the parties, become immediately due and payable without presentment, demand for payment, protest or other notice of any kind (all of which are expressly waived) upon the occurrence of any one or more of the following events (each an "**Event of Default**"):

(a) The occurrence of an Event of Default under and as defined in the Lender's Note, and the continuation of such Event of Default unremedied beyond any applicable grace period provided for in the Lender's Note;

(b) The occurrence of an Event of Default under and as defined in the Loan Agreement of even date herewith between Debtor and Secured Party, and the continuation of such Event of Default unremedied beyond any applicable grace period provided for in the Loan Agreement;

(c) The occurrence of an Event of Default under and as defined in the loan agreement of even date herewith between Debtor and Secured Party, and the continuation of such Event of Default unremedied beyond any applicable grace period provided for in such loan agreement;

(d) Failure by the Debtor to duly observe any covenant, condition or agreement of this Agreement, and the continuation of such failure unremedied for ten (10) days after notice of such failure is given by Secured Party to Debtor;

(e) The sale, conveyance, transfer, mortgage, lease or encumbrance of all or any portion of the Collateral, except in the ordinary course of business at market prices; and

(f) Debtor suffers or permits any lien, encumbrance, or security interest to arise or attach to the Collateral (other than liens in favor of the Secured Party and Permitted Encumbrances) that is not promptly removed or satisfied.

15. **Rights and Remedies**. If an Event of Default shall have occurred and be continuing, the Secured Party, without any other notice to or demand upon the Debtor, shall have in any jurisdiction in which enforcement hereof is sought, in addition to all other rights and remedies, the rights and remedies of a secured party under the applicable Uniform Commercial Code and any additional rights and remedies as may be provided to a secured party in any jurisdiction in which Collateral is located, including, without limitation, the right to take possession of the Collateral, and for that purpose the Secured Party may, so far as the Debtor can give authority therefore, enter upon any premises on which the Collateral may be situated and remove the same therefrom. Unless the Collateral is perishable or threatens to decline speedily in value or is of a type customarily sold on a recognized market, the Secured Party shall give to the Debtor at least five Business Days prior written notice of the time and place of any public sale of Collateral or of the time after which any private sale or any other intended disposition is to be made. The Debtor hereby acknowledges that five Business Days prior written notice of such sale or sales shall be reasonable notice. In addition, the Debtor waives any and all rights that it may have to a judicial hearing in advance of the enforcement of any of the Secured Party's rights and remedies hereunder, including, without limitation, its right following an Event of Default to take immediate possession of the Collateral and to exercise its rights and remedies with respect to this Agreement.

16. **Standards for Exercising Rights and Remedies**. To the extent that applicable law imposes duties on the Secured Party to exercise remedies in a commercially reasonable manner, the Debtor acknowledges and agrees that it is not commercially unreasonable for the Secured Party: (a) to fail to incur expenses reasonably deemed significant by the Secured Party to prepare Collateral for disposition or otherwise to fail to complete raw material or work in process into finished goods or other finished products for disposition, (b) to fail to obtain third party consents for access to Collateral to be disposed of, or to obtain or, if not required by other law, to fail to obtain governmental or third party consents for the collection or disposition of Collateral to be collected or disposed of, (c) to fail to exercise collection remedies against account debtors or other persons obligated on Collateral or to fail to remove liens or encumbrances on or any adverse claims against Collateral, (d) to exercise collection remedies against account debtors and other persons obligated on Collateral directly or through the use of collection agencies and other collection specialists, (e) to advertise dispositions of Collateral through publications or media of general circulation, whether or not the Collateral is of a specialized nature, (f) to contact other persons, whether or not in the same business as the Debtor, for expressions of interest in acquiring all or any portion of the Collateral, (g) to hire one or more professional auctioneers to assist in the disposition of Collateral, whether or not the collateral is of a specialized nature, (h) to dispose of Collateral by using Internet sites that provide for the auction of assets of the types included in the Collateral or that have the reasonable capability of doing so, or that match buyers and sellers of assets, (i) to dispose of assets in wholesale rather than retail markets, (j) to disclaim disposition warranties, (k) to purchase insurance or credit enhancements to insure the Secured Party against risks of loss, collection or disposition of Collateral or to provide to the Secured Party a guaranteed return from the collection or disposition of Collateral, or (l) to the extent deemed appropriate by the Secured Party, to obtain the services of other brokers, investment bankers, consultants and other professionals to assist the Secured Party in the collection or disposition of any of the Collateral. The Debtor acknowledges that the purpose of this Section is to provide non-exhaustive indications of what actions or omissions by the Secured Party would fulfill the Secured Party's duties under the applicable Uniform Commercial Code or any other relevant jurisdiction in the Secured Party's exercise of remedies against the Collateral and that other actions or omissions by the Secured Party shall not be deemed to fail to fulfill such duties solely on account of not being indicated in this Section. Without limitation upon the foregoing, nothing contained in this Section shall be construed to grant any rights to the Debtor or to impose

any duties on the Secured Party that would not have been granted or imposed by this Agreement or by applicable law in the absence of this Section.

17. **No Waiver by Secured Party, etc**. The Secured Party shall not be deemed to have waived any of its rights and remedies in respect of the Obligations or the Collateral unless such waiver shall be in writing and signed by the Secured Party. No delay or omission on the part of the Secured Party in exercising any right or remedy shall operate as a waiver of such right or remedy or any other right or remedy. A waiver on any one occasion shall not be construed as a bar to or waiver of any right or remedy on any future occasion. All rights and remedies of the Secured Party with respect to the Obligations or the Collateral, whether evidenced hereby or by any other instrument or papers, shall be cumulative and may be exercised singularly, alternatively, successively or concurrently at such time or at such times as the Secured Party deems expedient.

18. **Suretyship Waivers by Debtor**. The Debtor waives demand, notice, protest, notice of acceptance of this Agreement, notice of loans made, credit extended, Collateral received or delivered or other action taken in reliance hereon and all other demands and notices of any description. With respect to both the Obligations and the Collateral, the Debtor assents to any extension or postponement of the time of payment or any other indulgence, to any substitution, exchange or release of or failure to perfect any security interest in any Collateral, to the addition or release of any party or person primarily or secondarily liable, to the acceptance of partial payment thereon and the settlement, compromising or adjusting of any thereof, all in such manner and at such time or times as the Secured Party may deem advisable. The Secured Party shall have no duty for the collection or protection of the Collateral or any income from the Collateral, the preservation of rights against prior parties, or the preservation of any rights pertaining to this Agreement. The Debtor further waives any and all other suretyship defenses.

19. **Marshalling**. The Secured Party shall not be required to marshal any present or future collateral security (including but not limited to the Collateral) for, or other assurances of payment of, the Obligations or any of them or to resort to such collateral security or other assurances of payment in any particular order, and all of its rights and remedies hereunder and in respect of such collateral security and other assurances of payment shall be cumulative and in addition to all other rights and remedies, however existing or arising. To the extent that it lawfully may, the Debtor hereby agrees that it will not invoke any law relating to the marshalling of collateral which might cause delay in or impede the enforcement of the Secured Party's rights and remedies under this Agreement or under any other instrument creating or evidencing any of the Obligations or under which any of the Obligations is outstanding or by which any of the Obligations is secured or payment thereof is otherwise assured, and, to the extent that it lawfully may, the Debtor hereby irrevocably waives the benefits of all such laws.

20. **Proceeds of Dispositions; Expenses**. The Debtor shall pay to the Secured Party on demand any and all expenses, including reasonable attorneys' fees and disbursements, incurred or paid by the Secured Party in protecting, preserving, or enforcing the Secured Party's rights and remedies under or in respect of any of the Obligations or any of the Collateral. After deducting all of said expenses, the residue of any proceeds of collection or sale or other disposition of Collateral shall, to the extent actually received in cash, be applied to the payment of the Obligations in such order or preference as the Secured Party may determine, proper allowance and provision being made for any Obligations not then due. Upon the final payment and satisfaction in full of all of the Obligations and after making any payments required by the applicable Uniform Commercial Code, any excess shall be returned to the Debtor. In the absence of final payment and satisfaction in full of all of the Obligations, the Debtor shall remain liable for any deficiency.

21. **Overdue Amounts**. Until paid, all amounts due and payable by the Debtor hereunder shall be a debt secured by the Collateral and shall bear, whether before or after judgment, interest at the rate of interest set forth in the Lender's Note.

22. **Governing Law; Consent to Jurisdiction**. THIS AGREEMENT IS INTENDED TO TAKE EFFECT AS A SEALED INSTRUMENT AND SHALL BE GOVERNED BY, AND CONSTRUED IN ACCORDANCE WITH, THE LAWS OF THE STATE OF SOUTH CAROLINA, except to the extent that the Uniform Commercial Code provides for the law of a different state to govern the perfection and priority of the security interests granted hereunder. The Debtor agrees that any action or claim arising out of any dispute in connection with this Agreement, any rights or obligations hereunder or the performance or enforcement of such rights or obligations may be brought in the courts of the State of South Carolina, or any Federal court sitting in such jurisdictions, and consents to the non-exclusive jurisdiction of such courts and to service of process in any such suit being made upon the Debtor by mail at the address set forth in the Loan Agreement. The Debtor hereby waives any objection that it may now or hereafter have to the venue of any such suit or any such court or that such suit is brought in an inconvenient court.

23. **Waiver of Jury Trial**. THE DEBTOR WAIVES ITS RIGHT TO A JURY TRIAL WITH RESPECT TO ANY ACTION OR CLAIM ARISING OUT OF ANY DISPUTE IN CONNECTION WITH THIS AGREEMENT, ANY RIGHTS OR OBLIGATIONS HEREUNDER OR THE PERFORMANCE OR ENFORCEMENT OF ANY SUCH RIGHTS OR OBLIGATIONS. Except as prohibited by law, the Debtor waives any right which it may have to claim or recover in any litigation referred to in the preceding sentence any special, exemplary, punitive or consequential damages or any damages other than, or in addition to, actual damages. The Debtor (i) certifies that neither the Secured Party nor any representative, agent or attorney of the Secured Party has represented, expressly or otherwise, that the Secured Party would not, in the event of litigation, seek to enforce the foregoing waivers or other waivers contained in this Agreement and (ii) acknowledges that, in entering into the Debtor Loan Agreement and the other Loan Documents to which the Secured Party is a party, the Secured Party is relying upon, among other things, the waivers and certifications contained in this Section.

24. **Waiver of Pre-Seizure Hearing**. In the event the Secured Party seeks to take possession of any of the Collateral by replevin, claim and delivery, or other court process, the Debtor hereby (i) **GRANTS THIS WAIVER OF HEARING PRIOR TO IMMEDIATE POSSESSION**; and (ii) irrevocably waives, to the extent permitted by law, any bonds, and any surety or security relating thereto, required by any statute, court rule or otherwise as an incident to such possession and any demand for possession of the Collateral prior to the commencement of any suit or action to recover possession thereof.

25. **Miscellaneous**. The headings of each section of this Agreement are for convenience only and shall not define or limit the provisions thereof. This Agreement and all rights and obligations hereunder shall be binding upon the Debtor and its successors and assigns, and shall inure to the benefit of the Secured Party and its successors and assigns. If any term of this Agreement shall be held to be invalid, illegal or unenforceable, the validity of all other terms hereof shall in no way be affected thereby, and this Agreement shall be construed and be enforceable as if such invalid, illegal or unenforceable term had not been included in this Agreement. The Debtor acknowledges receipt of a copy of this Agreement.

26. **Certain Defined Terms**. All terms defined in the applicable Uniform Commercial Code and used in this Agreement shall have the same definitions in this Agreement, unless otherwise specified. Capitalized terms not otherwise defined herein or in the Uniform Commercial Code shall have the meanings ascribed to them in the Debtor Loan Agreement.

The following language is hereby incorporated by reference into all mortgages, security agreements, assignments and any other loan documents which grant to Lender's a lien on property or property rights of the Borrower or any third party:

The Loan secured by this lien was made under a United States Small Business Administration (SBA) nationwide program which uses tax dollars to assist small business owners. If the United States is seeking to enforce this document, then under SBA regulations:

a) When SBA is the holder of the Note, this document and all documentation evidencing or securing this Loan will be construed in accordance with federal law.

b) Lender or SBA may use local or state procedures for purposes such as filing papers, recording documents, giving notice, foreclosing liens, and other purposes. By using these procedures, SBA does not waive any federal immunity from local or state control, penalty, tax or liability. No Borrower or Guarantor may claim or assert against SBA any local or state law to deny any obligation of Borrowers, or defeat any claim of SBA with respect to this Loan.

Any clause in this document requiring arbitration is not enforceable when SBA is the holder of the Note secured by this instrument.

IN WITNESS WHEREOF, intending to be legally bound, the Debtor has caused this Agreement to be duly executed in its name and its seal affixed hereto as of the date first above written.

Witnesses:

DEBTORS:

DPX TECHNOLOGIES, LLC (Co-Borrower)
a South Carolina limited liability company

By: _____
William E. Brewer
Its: Member

[SEAL]

DPX LABS, LLC (Co-Borrower)
a South Carolina limited liability company

By: _____
William E. Brewer
Its: Manager

[SEAL]

_____
William E. Brewer (Guarantor)

DPX Labs, LLC
Fixed Asset Listing
July 31, 2015

Item	Purchase Date	Purchase Description
000563	03/05/2014	Service Installation & Training, including the Tra
001 - H-841-10 5 Step Ladder	02/24/2012	5 Step Ladder w/10" Top Step
002 H-1188	02/22/2012	Two Tier Carton Stand
0020 901356 Chair, Ruzzi MI (3)	05/08/2013	901356 Chair, Ruzzi MI - 3
003 H-1534	02/22/2012	96x48x72" Wide Span Storage Rack
004 H-1532	02/22/2012	96x24x72" Wide Span Storage Rack
005 H-1532-Add	02/22/2012	96x24 Addl Shelf For 96x24x72
006 H-1105AT	02/22/2012	36x18x72 Tan Storage Cabinet
007 36 1/16" x 72 1/16"	02/22/2012	16 ga. 304 s/s tops 36-1/16" x 72-1/16"
008 36" x 84"	02/22/2012	16 ga. 304 s/s tops 36" x 84"
009 30" x 60"	02/22/2012	16 ga. 304 s/s tops 30" x 60"
010 36" x 80"	02/22/2012	16 ga. 304 s/s tops 36" x 80"
011 12" x 30"	02/22/2012	14 ga. s/s panels 12" x 30" w/ 8ea. mount holes
012 3-In-1 Hand Truck	02/27/2012	3-In-1 Hand Truck
013 HP Pavilion Workstation	06/10/2014	HP Pavilion Model 500-29T and C3Z93AA
014 Edgeport-2 USB/RS232 conver	07/10/2014	014 Edgeport-2 USB to 2 port RS-232, DB-9 converte
04670722000	03/16/2015	Kenmore 16.7 cu. ft. Freezerless Refrigerator
1 Lenovo Flex 2 15.6" Labtop	01/12/2015	1 Lenovo Flex 2 15.6" Labtop
1 mL Frit Setting Tool (Build a	05/03/2013	1 mL Frit Setting Tool (Build and Design)
199030	06/18/2015	FVK II Solutions
2-Cavity Blister Tub Sealer	04/04/2014	2-Cavity Blister Tub Sealer
235964	06/18/2015	50µL Nested Clear NSterile NFilter Tips
2372026	05/13/2015	Computer: ACER C15-4460/8gb/2tb/nt
44648 Worktable (2)	07/11/2013	44648 Worktable
500110	03/05/2014	Micro10x Robotic Dispenser, includes manual/robot
550001	03/05/2014	VaryVac_M - Manually loaded vacuum nest with auto-
550100	03/05/2014	2.5 US Gal. Waste carboy, with quick-disconnect fi
61050-01	06/18/2015	Assy, Pedestal NTR
61051-01	06/18/2015	Assy, Pedestal MTP
61052-01	06/18/2015	Reagent Trough Pedestal 5x50 mL
61053-01	06/18/2015	Assy, Pedestal DWP-std
61055-02	06/18/2015	Pedestal, MTP - Gripper Stack
61668-237	06/18/2015	Open NIMBUS96 Gripper 9+2-DISCOUNTED
63222-02	06/18/2015	NIMBUS LAPTOP COMPUTER
65619 Workbench	07/11/2013	65619 Workbench
8968306	05/13/2015	Brother Wireless J485 InkJet Printer
93830-01	06/18/2015	NVK Consumables
9551MS-BK Chair 22.5-32.5" Non	04/17/2014	9551MS-BK Chair 22.5-32.5" Non-ESD Cloth
Agilent 1200 High Performance A	05/20/2015	Agilent 1200 High Performance Autosampler (G1367C)
APCI Probe - TSQ Vantage	10/24/2013	APCI Probe for Use on Ion Max Source for TSQ Vanta
Capill-580 MCRN, ASYM ION ST-2	11/01/2013	Capillary-580 MCRN, ASYM ION ST (2)
DPX - VP710D2	01/23/2015	Rotary Magnetic Tumble Stirrer
DPX - VP710D2-2	01/23/2015	VP710D2 accessory SBS deck to hold 96 res
DPX - VP750-ECON-6	01/23/2015	Economy Tumble Bubble Paddle Reservoir
DPX - VP782N-6	01/23/2015	NdFeB (48MGO) Magnetic Tumble Stir Discs
DPX - VP792D	01/23/2015	Disposable Nalgene Polypropylene Reservoirs
DPX 4014	01/21/2015	Single Channel VIOFLO Pipette 50-1250uL
DPX 4024	01/21/2015	8 Channel Vision Pipette 50-1250uL
DPX 6230	01/30/2015	DPX 6230 Three Position Stage
DPX G1312B	03/24/2015	Agilent 1200 SL Binary Pump (G1312B)
DPX G1316B	03/24/2015	Agilent 1200 Column Compartment (G1316B)
DPX G1379	03/24/2015	Agilent Degasser (G1379)
DPX GER 093711-044-00	12/31/2014	100uL "X-type" gastight syringe for MPS 3 22g
DPX GER 093711-046-00	02/13/2015	100 µL LC syringe kit for for MPS 3
DPX GER 093731-003-00	02/13/2015	AUX valve drive for MPS injection valves (Valco va
DPX GER 093731-015-00	02/13/2015	Fast Wash Station for two solvents for MPS
DPX GER 093731-034-00	02/13/2015	Sample loop kit, 20 uL Valco (Cheminert), Includes
DPX GER 093731-120-00	02/13/2015	6 port cheminert injection valve 0.25 mm bore, 500
DPX GER 093731-154-00	02/13/2015	Sample loop kit, 2 uL Valco (Cheminert), Includes
DPX GER G1312B	12/31/2014	GER G1312B: 1260 Infinity binary pump
DPX GER G1312B-001	12/31/2014	GER G1312B-001
DPX GER G1312B-002	12/31/2014	GER G1312B-002
DPX GER G1312B-004	12/31/2014	Agilent Lab Advisor
DPX GER G1312B-030	12/31/2014	Active seal wash option
DPX GER G1312B-031	12/31/2014	Solvent Selection Valve Option

Item	Purchase Date	Purchase Description
DPX GER G1312B-500	12/31/2014	LAN Communications Interface
DPX GER G1316C	12/31/2014	1290 Infinity thermostatted column compartment.
DPX GER G1316C-058	12/31/2014	Valve drive for TCO SL Plus
DPX GER G4225A	12/31/2014	1260 Infinity High Performance Degasser
DPX GER G4232A	12/31/2014	2pos/10port micro valve head 600bar1
DPX GER G4232A-003	02/13/2015	Low dispersion capillary kit, 0.12mm ID
DPX GER GS 1600-01	02/13/2015	Installation
DPX GER GS 1900-01	02/17/2015	Shipping
DPX H-1135-STEEL (2)	01/16/2015	DPX H-1135-STEEL (2)
DPX HRB203 Milligram Balance	01/01/2015	Tree HRB 203 High Resolution Economy Milligram Bal
DPX M1424757	01/30/2015	Air Science, FLOW-48, Vertical Laminar Flow Cabine
DPX6001	01/30/2015	DPX6001 VIAFLO 96 Base Unit (Qty: 2)
DPX6104	01/30/2015	DPX6104 50-1250 96 Channel Pipetting Head (Qty: 2)
DPX6205	02/04/2015	DPX6205 Standard Plate Holder (QTY: 4)
DPXAgilent 6550	02/04/2015	Agilent 6550 iFunnel Accurate-Mass Quadrupole Time
DPXItrogen Gas Generator for Ag	01/30/2015	NEW Nitrogen Gas Generator for Agilent, Waters & T
DPXPELICANCASE	06/18/2015	Pelican Case for Viaflo 96
INSTALL16	03/05/2014	NIMBUS INSTALLATION
MEC-1544	03/05/2014	Dispense manifold, 12-channel
NPN-1	03/05/2014	Custom Vacuum Nest Inserts/Standoffs to host DPX T
NPN-2	10/02/2014	VaryVac to Micro10x Alignment Base
Pipettor - MFG Part #4624, 4443	11/01/2013	Pipettor - MFG Part #4624, 4443
S-2056	06/18/2013	VorTemp 56 Micro Shaking Incubator
S/N TQ02395	05/03/2013	Mass Spectrometer
Standard Pipette Tip Holder	10/01/2013	Standard Pipette Tip Holder
Surface - BKemnitzer	01/31/2014	Microsoft Surface + Accessories
Tip Mold - Hamilton	07/15/2011	Tip Mold - Hamilton: HT-1000-WB/HT-300-WB
Tooling	05/10/2013	Tooling: Multi-cavity mold - 8000pc / week
VP 710D2-1	05/10/2013	VP 710D2 accessory deck to hold VP 756B bubble pad
VP 756A	05/10/2013	Bubble Paddle Reservoir, polypropylene, Includes 1
VP710D2		Rotary Magnetic Tumble Stirrer, 1250 Max RPM, 25mm

United States Patent **6,566,145**
Brewer **May 20, 2003**

Disposable pipette extraction

Abstract

The present invention is a disposable apparatus for the rapid, low-volume solid phase extraction of analytes from a variety of sources. The apparatus of the present invention is configured as a pipette tip and contains a loosely confined stationary phase. The mobility of the stationary phase particles enables rapid mixing and equilibration with a sample solution during agitation. The analyte may thereby be extracted in less time with less solvent, removing the need for a separate concentration step.

Inventors: *Brewer*; **William E** (Columbia, SC)
Family ID: **29272499**
Appl. No.: **09/780,885**
Filed: **February 9, 2001**

Current U.S. Class:	**436/178**; 210/661; 422/562; 73/864.01
Current CPC Class:	B01L 3/0275 (20130101); G01N 1/405 (20130101); Y10T 436/255 (20150115); G01N 2030/062 (20130101); G01N 2030/009 (20130101)
Current International Class:	B01L 3/02 (20060101); G01N 1/34 (20060101); G01N 30/00 (20060101); G01N 30/06 (20060101); B01L 003/02 ()
Field of Search:	;436/178,180 ;422/100,101 ;73/864.01 ;210/661

References Cited [Referenced By]

U.S. Patent Documents

4151089	April 1979	Ito
5042502	August 1991	Guirguis
5171537	December 1992	Wainwright et al.
5368729	November 1994	Stefkovich et al.

5437979	August 1995	Rampal et al.
5443734	August 1995	Fetner et al.
5512168	April 1996	Fetner et al.
5529694	June 1996	Strickler
5595653	January 1997	Good et al.
5660792	August 1997	Koike
5849249	December 1998	Jones, Jr. et al.
5874004	February 1999	DeWitt
5888409	March 1999	Morsiani et al.
6048457	April 2000	Kopaciewicz et al.
6117394	September 2000	Smith

Other References

ZipTip Pippette Tips at <<http://www.millipore.com/catalogue.nsf/docs/C5737>> is an online catalog of biological filtration devices. Here, pipette tips having a polymer-bound extractions medium are disclosed..

Primary Examiner: Ludlow; Jan
Attorney, Agent or Firm: Mann; Michael A. Centioni; Sara A. Nexsen Pruet Jacobs & Pollard, LLC

Parent Case Text

PRIORITY CLAIM

The applicant claims the benefit of the filing date of provisional application No. 60/181,340 filed Feb. 9, 2000.

Claims

What is claimed is:

1. A pipette tip for solid phase extraction comprising: a housing having a proximal end with a lower opening adapted for the passage of liquid and a distal end with an upper opening dimensioned to fit on the end of a pipettor; a first frit inside said housing and above said lower opening; a second frit inside said housing and between said first frit and said upper opening; and a plurality of adsorptive particles inside said housing and confined between said first frit and said second frit, wherein said adsorptive particles and said second frit are spaced apart so as to form a void therebetween, and wherein said void is dimensioned so that said adsorptive particles can travel freely within said void allowing for thorough mixing between said adsorptive particles and a sample solution when said sample solution is in said void.

2. The pipette tip as recited in claim 1, wherein said void is dimensioned so that a quantity of air can be drawn in through said pipette tip for agitation of said adsorptive particles and said sample solution.

3. The pipette tip as recited in claim 1, further comprising means for agitating that is operatively connected to said housing.

4. The pipette tip as recited in claim 1, wherein said first frit is permeable by gases and liquids, but not solids.

5. The pipette tip as recited in claim 1, wherein said first frit is selected from the group consisting of sintered glass plug, glass wool plug, porous polymer plug, and metal screen.

6. The pipette tip as recited in claim 4, wherein said second frit is permeable by gases, but not liquids or solids.

7. The pipette tip as recited in claim 4, wherein said second frit is selected from the group consisting of sintered glass plug, porous polymer plug, and semi-permeable membrane.

8. The pipette tip as recited in claim 1, wherein said housing comprises a material selected from the group consisting of polyethylene, polypropylene, polyethylene-terephthalate, and polytetrafluoroethylene.

9. The pipette tip as recited in claim 1, wherein said adsorptive particles comprise a material selected from the group consisting of silica, derivitized silica, polystyrene-divinylbenzene copolymer, functionalized polystyrene-divinylbenzene copolymer, and activated carbon.

10. The pipette tip as recited in claim 1, wherein said housing comprises a material selected from the group consisting of polyethylene, polypropylene, polyethylene-terephthalate, and polytetrafluoroethylene.

11. The pipette tip as recited in claim 1, wherein said adsorptive particles comprise a material selected from the group consisting of silica, derivitized silica, polystyrene-divinylbenzene copolymer, functionalized polystyrene-divinylbenzene copolymer, and activated carbon.

12. A process for extracting an analyte from a liquid, said process comprising the steps of: providing a pipette tip with a housing having a lower opening adapted for the passage of liquid and a distal end with an upper opening dimensioned to fit on the end of a pipettor, a first frit inside said housing and above said lower opening, and a plurality of adsorptive particles inside said housing and confined between said first frit and said upper opening, wherein said adsorptive particles and said upper opening are spaced apart so as to form a void therebetween, and wherein said void is dimensioned so that said adsorptive particles can travel freely within said void allowing for thorough mixing between said adsorptive particles and a sample solution when said sample solution is in said void; attaching said pipette to a pipettor; drawing a sample liquid into said pipette tip so that said liquid is in physical contact with said adsorptive particles; agitating said liquid sample in said pipette tip, wherein said agitating step is selected from the group consisting of shaking said liquid sample, vortexing said liquid sample, and drawing air into said liquid sample; expelling said sample liquid from said pipette tip; adding extraction solvent into said pipette tip so said extraction solvent is in physical contact with said adsorptive particles; and expelling said extraction solvent from said pipette tip.

13. The process for extracting an analyte from a liquid as recited in claim 12, wherein said process is conducted without a concentration step.

14. The process for extracting an analyte from a liquid as recited in claim 12, said process further

comprising the step of disposing of said pipette tip.

15. The process for extracting an analyte from a liquid as recited in claim 12, said process further comprising the step of analyzing said extraction solvent.

16. The process for extracting an analyte from a liquid as recited in claim 12, wherein said first frit is permeable by gases and liquids, but not solids.

17. The process for extracting an analyte from a liquid as recited in claim 12, wherein said first frit is selected from the group consisting of sintered glass plug, glass wool plug, porous polymer plug, and metal screen.

18. The process for extracting an analyte from a liquid as recited in claim 12, wherein said housing comprises a material selected from the group consisting of polyethylene, polypropylene, polyethylene-terephthalate, and polytetrafluoroethylene.

19. The process for extracting an analyte from a liquid as recited in claim 12, wherein said adsorptive particles comprise a material selected from the group consisting of silica, derivitized silica, polystyrene-divinylbenzene copolymer, functionalized polystyrene-divinylbenzene copolymer, and activated carbon.

Description

FIELD OF THE INVENTION

The present invention relates to an apparatus for rapid, disposable, low volume solid phase extraction and to a method for using such an apparatus.

DISCUSSION OF BACKGROUND

Solid Phase Extraction (SPE) has become popular in sample preparation. The main advantage of SPE is that less solvent is required as compared to traditional liquid extraction. In SPE, a cartridge composed of plastic is used to store the adsorptive particle of stationary phase as well as to provide a sample reservoir. The cartridge is placed on a vacuum manifold, and the vacuum is used to pull sample and solvent through the stationary phase. The stationary phase is first washed and activated by addition of various solvents (approximately 1-3 mL each) in a conditioning step. The conditioning step is also essential to prevent channeling, a process in which the sample components pass though the stationary phase packing without actually interacting with the adsorptive particles of the stationary phase in a chemically-meaningful manner.

The sample matrix is subsequently added to the cartridge, and the matrix is passed slowly though the stationary phase to allow the analyte to interact with the stationary phase. Several types of analyte-stationary phase interactions are possible, such as adsorption and partitioning. After the sample matrix has passed through the cartridge to waste, a wash step is performed to remove compounds of the sample matrix. The final step is elution of the analytes. A clean test tube is first placed under the SPE cartridge, then elution solvent (1-2 mL) is added to remove the analytes from the stationary phase.

After the extraction procedure, a concentration step is performed to improve the sensitivity of analysis. The solvent from the extract (1-2 mL) is evaporated using nitrogen gas flow and heat. A small volume of

solvent (0.10-0.20 mL) is then added to the test tube, and the test tube is vortexed to dissolve the extracted analytes. The solution is subsequently transferred to a clean vial for analysis by gas chromatography (GC) or high performance liquid chromatography (HPLC).

Although SPE has helped to make sample preparation faster and easier, the concentration step by itself takes several minutes to perform, with the entire extraction time taking over 20 minutes. Another drawback to SPE is that significant solvent volumes are still required (at least 5 mL total). An extraction procedure that significantly reduces the extraction time and reduces solvent volume will have a significant impact in analytical preparation methods.

There have been numerous attempts to remedy the foregoing deficiencies in SPE. A notable modification of this technology is disclosed in U.S. Pat. No. 6,048,457 issued to Kopaciewicz et al. This patent discloses a method of SPE that uses a polymer-matrix bound sorbent material. In one embodiment, a porous polymer matrix entraps particles of adsorbent material and is cast-in-place inside a pipette tip. In a contrasting example, sorbent particles are immobilized between two porous frits. This invention provides an effective platform for micromass handling. Unfortunately, the matrix-bound particles are unable to achieve maximum contact with the sample solution due to minimal exposed surface area and severely restricted mobility of the particles. As a result, the efficiency of the solid-liquid equilibrium leaves much room for improvement.

Therefore, there still exists a substantial need for a chemical extraction device that adequately overcomes the problems existing in preparing chemical samples for chromatographic separation techniques.

SUMMARY OF THE INVENTION

According to its major aspects and broadly stated, the present invention is a device for rapid, disposable, low-volume solid phase extraction of analytes from various fluids to be tested. The term fluid refers to liquids and to semi-liquids. In particular, the present invention relates to a pipette tip which contains adsorptive particles of a solid stationary phase that are loosely confined inside the tip. An attached pipettetor draws the sample into the pipette tip. The present invention is also a method for using the foregoing pipette tip to extract an analyte. Maximum contact and thorough mixing between the stationary phase and the analyte solution is accomplished by agitating them (shaking, inverting repeatedly, vortexing or by drawing air into the pipette). Agitation insures rapid equilibration and, thus, efficient and rapid adsorption of the analyte.

Other features and advantages of the present invention will be apparent to those skilled in the art from a careful reading of the Detailed Description of a Preferred Embodiment accompanied by the following drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

In the drawings,

FIG. 1 is a front view of a typical pipette tip which can be used with a pipettor.

FIG. 2 is a detailed cross-sectional view along line 2 of FIG. 1 of a preferred embodiment of the present invention;

FIG. 3 is a chromatogram of a drug mixture extracted from serum using the disposable pipette tip of the present invention;

FIG. 4 is a chromatogram of an organochlorine pesticide mixture extracted from a mixture of acetonitrile and water using the disposable pipette tip of the present invention; and

FIG. 5 is a chromatogram of an organophosphorous pesticide mixture extracted from a mixture of acetonitrile and water using the disposable pipette tip of the present invention.

DETAILED DESCRIPTION OF A PREFERRED EMBODIMENT

The present invention is a disposable pipette tip extraction apparatus for use in rapid, disposable, and low-volume solid phase extraction of analytes.

With reference to FIG. 1 and FIG. 2, a typical pipette tip 10 is shown having a proximal end 12 with lower opening 14 and a distal end 20 with upper opening 16. Pipette tip 10 can be made of any inexpensive material or commodity plastic, but is preferably made from a polyolefin, and most preferably made from polyethylene, polypropylene, polyethylene-terephthalate, or polytetrafluoroethylene. The distal end 20 is configured to fit on the end of a standard laboratory pipettor. The proximal end 12 is configured to allow the passage of whatever fluid one wishes to sample with the pipettor. Moreover, the pipette tip is preferably conical in shape, with distal end 20 having a larger internal diameter than proximal end 12.

FIG. 2 shows a cross section of an embodiment of the present invention. Within the lumen of pipette tip 10 is first frit 24. The purpose of first frit 24 is to provide a permeable barrier which permits the unrestricted passage of fluids in either direction but does not allow the stationary phase material to pass through, thereby insuring that no loss of stationary material occurs. First frit 24 can be a sintered glass plug, a glass wool plug, a porous polymer plug, or a metal screen. A second frit 22 is located between first frit 24 and distal end 20. Second frit 22 is optional. The purpose of second frit 22 is to prevent the passage of either solids or fluids therethrough; this insures the retention of the stationary phase within and contamination of the pipette tip and the fluid below second frit 22, and prevents contamination of the pipettor by sample solution or solvents during the agitation step. Second frit 22 is preferably a sintered glass plug, a porous polymer plug, or a semi-permeable membrane

First frit 24 and second frit 22 are spaced apart so as to form a void therebetween; this void can function as a mixing chamber for the various components of the present invention. Confined in the void formed between first frit 24 and second frit 22 are adsorptive particles of stationary phase 18. Stationary phase 18 is selected to have an affinity for the desired analyte and may be any suitable material which may be used in standard solid phase extraction techniques. Preferably, stationary phase 18 is silica, derivitized silica, polystyrene-divinylbenzene copolymer, functionalized polystyrene-divinylbenzene copolymer, or activated carbon. The particles of stationary phase 18 may freely travel between first frit 24 and second frit 22 such as, for example, during agitation; free movement between first and second frits 24, 22, respectively, allows for maximum contact and thorough mixing with the sample fluid and rapid equilibration through agitation.

The present invention is also a method for using the foregoing pipette tip to extract an analyte from a sample solution.

Pipette tip 10 is attached to a standard laboratory pipettor, and a volume of sample liquid is drawn into pipette tip 10 so the sample liquid is in physical contact with the adsorptive particles of stationary phase 18. In order to achieve maximum contact and thorough mixing between stationary phase 18 and the sample fluid, the pipette tip and attached pipettor are agitated. This can be accomplished by shaking or

inverting repeatedly, but is accomplished preferably by vortexing with a common laboratory vortexer. Such agitation insures rapid equilibration and, thus, efficient and rapid adsorption of the analyte by stationary phase 18.

The sample liquid is then expelled to waste from pipette tip 10 through lower opening 14 in proximal end 12. Next, an optional wash step can be rapidly performed by drawing up wash solvent (e.g. water), agitating the resulting solvent/stationary phase mixture, and expelling the wash solution to waste. The analyte is then removed from stationary phase 18 by drawing a small volume of extraction solvent into pipette tip 10 and agitating as before. The resulting analyte solution is then expelled from pipette tip 10. It is notable that the solution may be immediately analyzed by conventional laboratory techniques, without the need for a concentration step; the efficiency of the equilibration insures that the analyte solution is of high concentration and may be analyzed directly. Pipette tip 10 may be removed from the pipettor and disposed of.

The present method is further described by the following examples.

EXAMPLE 1--DPX

A pipette tip (1 mL) was fitted with a frit at the bottom. Stationary phase (10 mg, Oasis.RTM. HLB, Waters Corp.) was added to the pipette tip (FIG. 2). The stationary phase was activated by adding methanol and water. Only 0.20 mL methanol was drawn into the pipette tip using a pipettor. The methanol was vortexed with stationary phase for 5 sec., then the methanol was delivered to a waste container. The activation was completed by drawing 0.20 mL water, vortexing for 5 sec., and delivering the water to waste. The serum sample (0.5 mg/L SPE drug mix) was extracted by drawing 0.50 mL serum into the tip. The sample was then vortexed for 20 sec. The serum was subsequently delivered to waste. The wash step was performed by drawing 0.50 mL water into the tip, vortexing for 10 sec., and delivering the water to waste. Elution of adsorbed analytes was performed by drawing 0.15 mL of 20% methanol in ethyl acetate, vortexing for 10 sec., and delivering the solution to a vial. This procedure was repeated with an additional 0.15 mL of elution solvent. A disposable glass pipette was then used to draw the extract solution, a few drops were sent to waste to remove unwanted water content, and the solution was transferred to a vial insert.

The extract was injected into a GC/MS instrument directly (FIG. 3), without any concentration step. The total extraction time took approximately 3 minutes to perform. This extraction is referred to as disposable pipette extraction (DPX). DPX provides a means for thoroughly and rapidly mixing the stationary phase with sample and solvent. The used pipette tip is subsequently disposed of, and a new one is added to the pipettor to begin subsequent extractions. The results are shown in FIG. 3 as follows: 1. Amphetamine; 2. Methamphetamine; 3. Meperidine; 4. Glutehimide; 5. Phencyclidine (PCP); 6. Methadone; 7. Methaqualone; 8. Amitriptyline; 9. Cocaine; 10. Imipramine; 11. Doxepin; 12. Desipramine; 13. Pentazocine; 14. Codeine; and 15. Oxycodone.

EXAMPLE 2--DPX

The same protocol was followed as described in Example 1, above, but the extraction was from 2 mL of a mixture of acetonitrile and water (65/35) and the organochlorine pesticide concentration was 1.0 ppm. The sample was first evaporated to less than 1 mL with nitrogen gas flow, then the sample was extracted by DPX in less than 5 minutes using 0.20 mL of organic solvent. A concentration step was not performed. The results are shown in FIG. 4 as follows: 1. .alpha.-BHC; 2. .alpha.-BHC; 3. .alpha.-BHC; 4. .alpha.-BHC; 5. Heptachlor; 6. Aldrin; 7. Heptachlor epoxide; 8. Endosulfan; 9. p,p'-DDE; 10. Dieldrin; 11. Endrin; 12. Endosulfan; 13. p,p'-DDD; 14. Endrin aldehyde; 15. p,p'-DDT; 16. Endosulfan

sulfate; and 17. Methoxychlor.

EXAMPLE 3--DPX

The same protocol as described in Example 1, above, except that the analyte was an organophosphorous pesticide mixture (1.0 ppm). The results are shown in FIG. 5 as follows: 1. Dichlorvos; 2. Mevinphos; 3. Ethoprophos; 4. Naled; 5. Phorate; 6. Demeton; 7. Diazinon; 8. Disulfoton; 9. Methyl azinphos; 10. Methyl parathion; 11. Ronnel; 12. Fenthion; 13. Chlorpyrifos; 14. Trichloronate; 15. Stirofos; 16. Prothiofos; 17. Merphos; 18. Fensulfothion; 19. Bolstar; and 20. Coumaphos.

It will be clear to those skilled in the art of analytical chemistry that many modifications and substitutions can be made without departing from the spirit and scope of the invention, which is defined by the appended claims.

* * * * *

United States Patent
Brewer

8,715,593
May 6, 2014

Pipette tips for extraction, sample collection and sample cleanup and methods for their use

Abstract

The present invention is a pipette tip device for extraction of liquid, semi-solid or solid solutions to be chemically analyzed and the methods for their use. The pipette tip extraction device contains a screen or filter at its lower narrow end to contain solid particulate matter and a barrier at its upper wide end. The optional upper frit is to be made of material that permits liquid solutions to flow through it. In addition to the barrier and screen, the pipette tip extraction device may contain solid-phase sorbent. Through the use of a removable cap, the pipette extraction tip may also serve as a sample collection container or tip in which samples can be delivered to the top of the tip (e.g., for direct collection of samples, including solid samples). A new method for DPX extraction using liquid-liquid-solid-phase extraction is also disclosed.

Inventors: **Brewer; William** (Columbia, SC)

Applicant:

Name	City	State	Country	Type
Brewer; William	Columbia	SC	US	

Family ID: 39710492
Appl. No.: 12/525,546
Filed: February 21, 2008
PCT Filed: February 21, 2008
PCT No.: PCT/US2008/054584
371(c)(1),(2),(4) Date: August 02, 2009
PCT Pub. No.: WO2008/103828
PCT Pub. Date: August 28, 2008

Prior Publication Data

Document Identifier	Publication Date
US 20100081209 A1	Apr 1, 2010

Related U.S. Patent Documents

Application Number	Filing Date	Patent Number	Issue Date
60902463	Feb 21, 2007		
60987578	Nov 13, 2007		

Current U.S. Class: **422/527**; 422/501; 422/511; 422/512; 422/513; 422/524; 422/525; 422/534; 422/535; 422/570; 73/864; 73/864.01; 73/864.11; 73/864.74

Current CPC Class: B01L 3/0275 (20130101); B01L 3/5029 (20130101); B01L 2300/044 (20130101); B01L 2300/0681 (20130101); B01L 2300/069 (20130101); B01L 2200/0631 (20130101); Y10T 436/255 (20150115)

Current International Class: B01D 15/00 (20060101); B01L 3/02 (20060101)

Field of Search: ;422/524-525,513,527,534-535,570,500-501,511-512 ;73/863.32,864,864.01,864.02,864.03,864.11,864.14,864.74

References Cited [Referenced By]

U.S. Patent Documents

5171537	December 1992	Wainwright et al.
5437979	August 1995	Rampal et al.
5496523	March 1996	Gazit et al.
5580529	December 1996	DeVaughn et al.
5833927	November 1998	Raybuck et al.
6143252	November 2000	Haxo et al.
6455325	September 2002	Tajima
6566145	May 2003	Brewer
6692703	February 2004	Shoji et al.
6761855	July 2004	Cook et al.
7227018	June 2007	Shoji et al.
7785466	August 2010	Smith
2003/0007897	January 2003	Creasey
2003/0039589	February 2003	Smith
2005/0045543	March 2005	Gjerde et al.
2005/0169800	August 2005	Bianca

Other References

Young, International Search Report, PCT/US08/54584, Jun. 27, 2008. cited by applicant.

Primary Examiner: Gordon; Brian R
Attorney, Agent or Firm: Kanos; Sara C. Nexsen Pruet, LLC

Parent Case Text

This application claims priority from U.S. Provisional Application Ser. No. 60/902,463, filed 21 Feb. 2007, and Ser. No. 60/987,578, filed 13 Nov. 2007, both of which are incorporated in their entirety herein.

Claims

The invention claimed is:

1. A pipette tip system, comprising: a positive or negative pressure device; an upper adaptor including an entry, wherein said entry is a pierceable membrane, wherein said pierceable membrane is nonporous and adapted to be pierced by said positive or negative pressure device, and wherein said upper adaptor forms an air tight seal with said positive or negative pressure device when said positive or negative pressure device pierces said pierceable membrane; a lower liquid permeable closure; a mixing chamber between said upper adaptor and said lower liquid permeable closure; and sorbent materials within said mixing chamber contained loosely enough so that said sorbent materials are moveable for mixing.

2. The pipette tip as recited in claim 1, wherein said upper adaptor includes grooves for transporting said pipette tip by an automated instrument.

3. The pipette tip in claim 1, wherein said adaptor is removable.

4. The pipette tip in claim 1, wherein said lower liquid permeable closure is a filtration membrane or screen.

5. The pipette tip in claim 1, wherein said positive or negative pressure device is a syringe having a needle.

6. The pipette tip in claim 1, further comprising an upper barrier below said adaptor.

7. A pipette tip system, comprising: a positive or negative pressure device; a pipette tip having an upper end and a lower end; an adaptor at said upper end including an entry having a pierceable membrane adapted to be pierced by said positive or negative pressure device, wherein said pierceable membrane is nonporous and forms an air tight seal with said positive and negative pressure device; a lower liquid permeable closure; a mixing chamber between said upper adaptor and said lower liquid permeable closure; and sorbent material contained loosely within said mixing chamber to allow for movement of said sorbent material.

8. The pipette tip in claim 7, further comprising an upper barrier below said adaptor.

9. The pipette tip in claim 7, wherein said positive or negative pressure device is a syringe having a needle.

Description

FIELD OF INVENTION

The present invention relates to an extraction and/or sample collection device used during sample preparation for chemical analysis.

BACKGROUND

Numerous types of sample preparation devices for chemical analysis have been developed that utilize pipette tips. The main advantage for using pipette tips is that they may be readily used with automated robotic liquid handlers. All of these available tips focus on the processing of liquid sample solutions that draw solutions in and out of the narrow bottom end of the pipette tips. Furthermore, all of these products are only amenable to low pressure applications. These tips cannot utilize small particle size sorbent with very high surface areas because these cause high back pressure. None of these inventions introduce solutions through the top wide opening end of the pipette tips.

Unfortunately, these inventions are not effective for analyzing viscous liquid sample solutions such as whole blood. None of these devices are capable of processing semi-solid samples such as waste water or tissue homogenate without some sort of sample pre-treatment such as protein precipitation, centrifugation or filtration. Furthermore, none of these pipette tip devices can be used for directly analyzing solid samples.

There exists a need for a pipette tip device that can be used to process liquid, semi-solid and solid sample solutions for chemical analysis.

SUMMARY OF INVENTION

According to its major aspects and broadly stated, the present invention is a pipette tip device for the extraction of liquid, semi-solid or solid solutions to be chemically analyzed and the methods for their use. The pipette tip extraction device contains a screen or filter at its lower narrow end to contain solid particulate matter and a barrier at its upper wide end. This barrier may be referred to as either a frit, an adaptor, a cap or a fitting and may be used interchangeably. The upper frit is to be made of material that permits liquid solutions to flow through it, and the upper frit may be optional and replaced with other fittings at the top of the DPX tip such as a removable cap, an adaptor, a luer lock fitting, a 2- or 3-way (or multiple port) valve fitting, an o-ring fitting, or septum cap.

The adaptor may also be designed to facilitate the movement of the pipette tip on robotic instrumentation. By incorporating grooves on the outside of the adaptor, a robotic gripper can pick up the tip and move it to various locations such as sample tubes and vials. The inside of the adaptor is designed to make an air tight seal with a syringe needle so that liquid solvents and gases can be aspirated and dispensed with relatively high pressures. Furthermore, the adaptor may contain a thin film to provide a seal to contain material inside of the tip prior to sample processing.

In addition to the barrier and screen, the pipette tip extraction device may contain solid-phase sorbent. In this case, the pipette extraction tip is an improved disposable pipette extraction (DPX) tip, with the ability to introduce solvent and sample solutions to its top wide opening end. The sorbent may be small particle size such as 5-20 microns in diameter, providing unsurpassed extraction efficiency. The use of such small particle size requires high pressure (HP), and these HP-DPX tips therefore require the use of adaptors to provide high pressure applications. The sorbent can also contain optional additives, for

example, buffers or buffer salts, to aid in the processing of the sample.

In addition to HP-DPX tips, other sorbents may be used to extract and remove unwanted sample matrices to "clean up" solutions, called DPX-CU. Other sorbent materials include antibodies for immunoaffinity applications (DPX-IA), functionalized groups for cation exchange (DPX-CX) and anion exchange (DPX-AX) applications, and porous materials for molecular weight separations (DPX-MW).

Another application of DPX is sample preparation for liquid chromatography (DPX-LCprep). These tips have a dual purpose of extracting unwanted compounds as well as filtering particulate matter. The DPX-LCprep tips are used to protect HPLC columns to extend their lifetimes and improve chromatographic separations.

Through the use of a removable cap, the pipette extraction tip may also serve as a sample collection container or tip (SC-Tip) in which samples can be delivered to the top of the tip. In this case the pipette extraction tip can be used in the field for direct collection of samples, including solid samples. The screen of the SC-Tip is used as a filtration medium to remove and filter particulate matter that may be present following the extraction process. This represents the only application of pipette extraction devices that may be used for directly processing solid samples.

A new method for DPX extraction using liquid-liquid-solid-phase extraction is also disclosed. This unique method of extraction will greatly extend analyte extractions and applications.

Other features and advantages of the present invention will be apparent to those skilled in the art from a careful reading of the Detailed Description of a Preferred Embodiment Accompanied by the following drawings.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 shows a cross-section schematic diagram of a HP-DPX tip.

FIG. 2 shows schematic diagrams of cross-sections of several caps or adaptors or fittings that can be used with a syringe device for connection to the top of the HP-DPX tip.

FIG. 3 shows a schematic diagram of a preferred embodiment of the pipette tip device.

FIG. 4 shows chromatograms (GC/MS) of DPX extracts of tetrahydrocannabinol (THC) and carboxy-THC (COOH-THC).

FIG. 5 shows a schematic diagram demonstrating the steps involved in DPX-LCprep.

FIG. 6 shows a schematic diagram of the tandem use of HP-DPX with DPX-LCprep to encompass rapid DPX extraction of analytes followed by the use of a disposable guard cartridge and filtration.

FIG. 7 shows GC/MS chromatograms of some common pesticides extracted from lettuce by DPX (B) and HP-DPX (A).

FIG. 8 shows a chromatogram (GC/MS) of basic drugs extracted from urine using DPX-CX.

FIG. 9 shows an HPLC chromatogram (with fluorescence detection) recorded following DPX-IA extraction of 20 ppb aflatoxin B.sub.1 (peak at 6.4 min) from corn meal.

FIG. 10 shows chromatograms (GC/MS) of blank stomach contents (feed) extracted by DPX with (A) and without (B) a clean up step by DPX-CU.

FIG. 11 shows a sample collection tip (SC-Tip) demonstrating the ability of the pipette extraction tips with adaptors to be used for field collection of samples as well as for sample processing.

DETAILED DESCRIPTION OF THE INVENTION

Numerous new sorbent materials have been recently developed, and use of these materials as sorbents vastly extend the capabilities of DPX methodology. Also, modifications to the DPX design must be incorporated in order to use many of these materials for DPX applications.

A major improvement to DPX is the development of high performance SPE by using small particle size sorbent material. These DPX tips are referred to as high performance DPX, or HP-DPX. The small particle size materials range up to 200 microns in diameter, preferably up to 100 microns, with a more preferred range of 5-40 microns and most preferred range of 5-20 microns. Use of these materials create higher back pressures and therefore require relatively higher pressures to perform extractions. HP-DPX tips can therefore not be simply press fitted onto standard pipettes to accomplish these extractions. Greater amounts of force then used with conventional pipettes for standard tips are required to move solutions in and out of the HP-DPX tips. For example, a 1 mL volume pipette (or syringe) can not be readily used with a 1 mL HP-DPX tip; instead, a 5 mL volume syringe or pipette device is required. For a 5 mL HP-DPX tip, a 10 or even 25 mL syringe device is required to effectively extract solutions.

Another feature that makes these HP-DPX tips unique is that the mixing of the solutions with many of these materials creates a "gel"; ie, the solution is homogenous and therefore provides unsurpassed extraction efficiencies and rapid equilibrations. The unique mixing of HP-DPX makes these particular materials ideally suited for DPX technology. One way of readily accomplishing this mixing is by aspirating air or another gas into the DPX tips, and this air flow causes the formation of small bubbles that cause a perturbation of the sample solution resulting in thorough mixing. This method of mixing is readily amenable to robotic liquid handlers.

In FIG. 1, a schematic of the HP-DPX tip is shown. The cross-section schematic diagram of an HP-DPX tip indicates an optional second frit (upper barrier, 22) composed of a liquid permeable membrane and loosely contained small particle size (such as 5 to 40 microns in diameter) sorbent material or other functionalized sorbent particles (18). The void (10) indicates the mixing chamber of DPX, and the bottom (12) of the tip contains an opening (14) with a proximate screen or frit (24) that contains very small openings or pores (such as 2 or 5 or 10 microns in diameter). The screen (24) may be composed of stainless steel, porous polymeric material, porous glass, porous ceramic, or other similar materials. The major improvements to this device is that different sorbent materials are disclosed and the upper frit (barrier) is made to be liquid permeable and is optional. Various sorbent materials include styrene divinyl benzene, sdvb (5-40 microns in diameter), and functionalized sdvb containing, for example, hydroxylated or sulfonated or aminated groups; primary secondary amine (psa); amino propyl or amino alkyl groups; alumina (basic, acidic or neutral); florisil; small particle size silica gel; C.sub.8, C.sub.18 and functionalized C.sub.8 or C.sub.18 material; Na.sub.2SO.sub.4, MgSO.sub.4 or CaSO.sub.4 (for drying); diatomaceous earth; sephadex; and polyethylene.

The use of small particle size sorbent material drastically improves recoveries and extraction efficiencies, but an adaptor or cap or fitting at the top of the DPX tip is required to securely connect these tips to perform the extractions. The adaptor or cap or fitting, which it can be referred to as either, may be

composed of plastic, metal or rubber, and can be disposable or reusable. With this tighter fit with the adaptor, the higher pressures don't cause the tips to "pop" off or become dislodged. In FIG. 2A, a schematic of a device including an adaptor (1a) that is a septum (A), a pipette tip (2a), sorbent or sample (3a), a liquid permeable closure (4a), and a void (7a) is shown. In FIG. 2B, a schematic of a device including an adaptor (1b) that is a Luer lock fitting, a seal (30b), a pipette tip (2b), sorbent or sample (3b), a liquid permeable closure (4b), a void (7b) or luer lock fitting adaptor (B) is shown. In this case, a syringe needle or syringe can be used to pierce the septum or connect directly to the adaptor, respectively. In FIG. 2C, a device is shown including, an adaptor (1c) that can also be used that forms to form a friction seal with a syringe needle, a pipette tip (2c), sorbent or sample (3c), a liquid permeable closure (4c), and a void (7c). In FIG. 2D, a schematic of a device is shown including an adaptor (1d) that is an o-ring seal that can be used to provide a seal for a syringe needle, a valve, Luer lock fitting or any other similar fitting, a pipette tip (2d), sorbent or sample (3d), a liquid permeable closure (4d), and a void (7d). Finally, in FIG. 2E a schematic of a device is shown including an adaptor (1e). The pipette tip (2e), sorbent or sample (3e), a liquid permeable closure (4e), and a void (7e). The the adaptor may contain a separate line for addition of liquid solvents (or gas) to the top of the DPX tip, and in this case a frit is shown to contain the sorbent (or sample) inside of the tip.

All of these adaptors can be modified to be used as transport adaptors for liquid robotic handlers. They may also contain a seal (30) or thin film so that they can be used as a cap to contain the contents inside of the tip, and the film can subsequently be penetrated in order to affect extraction methods. Also, all adaptors may be screw cap for easy closing and opening of the tips. In FIG. 3, a preferred embodiment of the adaptor is shown. The parts of the device include the adaptor (1), the pipette tip (2), the sorbent or sample (3), the void (7) and the liquid permeable closure (4). The adaptor is designed to form an air tight seal with a positive or negative pressure device, such as a syringe, and to facilitate movement for a robotic liquid handler as well as a cap to contain the contents within the pipette tip. Its key features are grooves (32) and ridges for securely attaching to a robotic instrument device, it can withstand high pressures for HP-DPX applications, it has a thin film (30) for containing material within the pipette tip device, and it has the ability to be removed as a cap for opening and closing the pipette tip device for sample collection (SC-Tip applications).

With these types of adaptors, the upper frit is not required because the septum or adaptor serves to contain the loose sorbent particles inside of the DPX tip. Hence, the upper frit is optional. Without the presence of the frit, it is much easier to add solutions such as elution solvent to the "top" of the DPX tip.

It has been determined that in some applications and methods, solutions added to the top provide better results. In the original DPX design, the upper barrier was indicated to be impermeable to liquid solutions, but this original design did not incorporate methods that added solutions to the top of the DPX tips. For example, elution using 5:1 hexanes-ethyl acetate for the analysis of THC (tetrahydrocannabinol, the active ingredient of marijuana) and its metabolites from whole blood is best performed when adding the elution solvent to the top of the DPX tip instead of drawing from the bottom. Elution from the bottom refers to drawing solution from the narrow end of the DPX tip (position 14 of FIG. 1), and elution from the top refers to adding elution solvent from the position of the adaptor (FIG. 2) at the "top" of the DPX tip.

In FIG. 4, GC/MS chromatograms of THC and its principle metabolite, COOH-THC, are shown following DPX extraction using elution solvent from the bottom (B) and elution from the top (A). (Note: all chromatograms in this application show intensity counts vs. time.) In this example, the upper barrier was made of a porous polymeric material that allows for liquid solutions to pass through without interferences. The peak representing COOH-THC is much greater in intensity with elution from the top. The necessity for adding elution to the top is more noticeable with the use of HP-DPX tips, where the

higher surface area causes retention of analyte when elution solvent is being passed back through the sorbent when eluting from the bottom, resulting in lower recoveries.

In order to facilitate the addition of solvent to the top of DPX tips, the adaptor uses a 3-way (or multi-) valve. This enables solvent to be readily added to the top without the need for disconnecting the DPX tips from the syringe device or pipette.

The use of other sorbent materials has been found to be useful for the detection of basic drugs. These compounds may be acidified and then extracted using cation exchange (DPX-CX) mechanisms. Similarly, this may be performed for the extraction and analysis of acidic drugs (or compounds), but these solutions are first made basic to make the compounds negatively charged, and these are subsequently extracted using DPX tips with anion exchange sorbent (DPX-AX).

Other DPX sorbent materials may also involve the use of various pore sizes for extracting and separating analyte based on molecular weight or size or shape. These DPX tips are referred to as DPX-MW.

Finally, DPX tips may also contain antibodies (or other proteins) immobilized on sorbent particles for the selective extraction of various analyte. These DPX tips are referred to as DPX-IA for immunoaffinity. The use of DPX-IA makes it possible to selectively extract a particular analyte in just seconds, and the use of the DPX design permits the analyte to rapidly mix and bind to the antibodies, separate the analyte from the sample matrix, and elute the analyte of interest for analysis. These DPX-IA tips may find wide practice for use in diagnostic tests, such as ELISA (enzyme linked immunosorbent assay), FPIA (fluorescene polarization immunoassay), EIA (enzymatic immunoassay), RIA (radioactive immunoassay), or other similar diagnostic techniques based on immunoaffinity technology. With DPX-IA, screening methods may be performed at a much faster rate than currently achieved using standard plates.

DPX methods in its original design focused on the extraction of analytes of interest. However, analysis of chemicals in various sample matrices is problematic due to high amounts of sample matrix components. These matrix compounds may cause interferences that obscure both qualitative and quantitative analytical data. An example is the presence of fatty acids in grain products or liver specimens. A new and improved feature of DPX is for the extraction of sample matrix interferences for use in cleanup steps, and these are referred to as DPX-CU (for cleanup). The DPX-CU procedure may use a weak or strong anion exchange (SAX) resin to remove fatty acid components. Other possible sorbent material for DPX-CU include polyamino, primary secondary amine (PSA), amino-alkyl groups, florisil, alumina (neutral, basic or acidic), silica gel, modified silica gel, molecular imprinted polymers, specific affinity type materials (such as antibodies, proteins or immobilized compounds to remove specific proteins), sephadex, varying pore size polymers, and anion exchange and cation exchange sorbent materials.

The methods used for DPX-CU are very fast, taking approximately 30 seconds or less to achieve, typically as short as 10 seconds. This rapid speed is due to the fact that the solutions are either drawn in and out of the tip or simply dispensed through the top of the tip to remove the interferences, so separate wash and elution steps are not required.

Another improvement to DPX is for its use as a device for preparation for HPLC analysis, referred to as DPX-LCprep. In HPLC analysis, the solutions to be injected into the chromatograph are first subjected to a filtration process. This is done to ensure the removal of particulate material that may clog and damage the HPLC column and instrumentation. Furthermore, a HPLC guard column is typically employed to prevent contamination of the chromatographic column. These guard columns contain solid-phase particles (sorbent) that are identical or very similar to the stationary phase of the HPLC column.

Although the use of guard columns protects the HPLC column, they inevitably cause some loss of resolution. The concept for the use of guard columns is that any compound that irreversibly binds to the stationary phase material of the HPLC column would first bind to the guard column (often referred to as the pre-column). After several analyses, the packing and the screens in the guard column are replaced in order to provide optimal chromatographic performance. Often, loss of performance is first noted and is used as an indicator or early warning that the guard column needs to be replaced. A much better approach is to incorporate a disposable guard column that protects the HPLC column and ensures each analysis is reproducible.

With DPX-LCprep, the tips are used to serve as both a disposable guard column and filtration device. The DPX-LCprep tips can be composed of the same type packing as the HPLC column, such as C.sub.18 or similar phase, and this ensures that any contaminants in the solution that may irreversibly bind to C.sub.18 is removed from the solution by binding to the sorbent contained in the DPX-LCprep tip. The bottom frit (screen) is liquid permeable and also acts as a filtration device, ensuring that particulate material is removed from the solutions. These DPX-LCprep tips are to be used by introducing the sample solutions to the top, thereby permitting the use of the bottom frit to act as a filtration device.

In this case, the DPX-LCprep sorbents are not to be mixed with the sample solution, but rather the solution is pushed through from the top. An example of the steps used for this process is shown in FIG. 5. Step A involves injecting the sample solution 52 with a syringe 50 into the DPX-LCprep tip 54. Step B involves dispensing the sample solution through the DPX-LCprep tip into an empty vial 56. The final step C involves collecting the clean and filtered sample solution in a vial 60, where sample matrix components are retained on the sorbent 58.

In FIG. 6, a schematic diagram of the tandem use of HP-DPX with DPX-LCprep to encompass rapid DPX extraction of analytes followed by the use of DPX-LCprep for a disposable guard column (or cartridge) and filtration is shown. This also demonstrates the feasibility for automation of these procedures. The schematic shows a HP-DPX tip 70 during the elution step; an adaptor seal 72 which connects the HP-DPX tip to a DPX-LCprep tip 74; conditioned LC media 76; a filtration screen 78; and the HPLC vial 80 for collecting the final "clean" eluent to be injected and analyzed. In this case, the adaptor 72 of the DPX-LCprep tip is designed to form a tight seal with another pipette tip and/or DPX tip. It is noteworthy that this procedure is readily automated.

Another key feature is that the DPX-LCprep tip may also act as a packed column which can be used to perform chromatography. Hence, the tip can be used as a chromatography medium which can be used to separate analytes for an inexpensive and portable method for chromatographic analysis. This is referred to as DPX-CHROM (for chromatography).

The method for performing DPX extractions may also be used to efficiently and rapidly perform liquid-liquid solid phase extraction. By mixing the sorbent with a particular solvent, the sorbent can subsequently be mixed with the sample solution. After a short equilibration time, the solution layers separate due to being immiscible and the sorbent settles into one of the layers (for example, the top organic layer). Choosing the solvent and sorbent to be immiscible with the sample solution will permit separation of phases without time-consuming centrifugation. Furthermore, there are no formations of emulsions that are common in liquid-liquid extractions. Also, the sorbent provides a clear indication of the separated layers. This liquid-liquid solid phase extraction is very unique and is ideally suited for use with DPX technology.

An advantage of the use of these adaptors is that they may also serve the purpose for permitting DPX tips to be used for sample collection containers (SC-Tips). The adaptors may serve as caps to contain samples

collected, especially of forensic interest. The SC-Tips may contain absorbent material for liquid solutions, as well as anticoagulant or coagulants (and preservatives) for the collection of whole blood or serum, respectively. The SC-Tip may also include material to bind and extract the analytes or the sample matrix components (such as the DPX-CU tips).

The SC-Tips may be used for collection and processing solid samples such as powders suspected to be drugs or explosives. During conventional forensic chemical analysis of drugs, the samples are first transferred from the evidence container into a suitable container for chemical analysis, such as a test tube. The solid sample is then dissolved in a solvent. Subsequently, the dissolved solution is filtered to remove diluents and fillers. Then the solution is transferred into a vial and its contents analyzed. All of these steps can be performed much more readily and efficiently if the samples are placed inside of the SC-Tip. Through manual processing or automation, the contents inside the SC-Tip can be processed with solvent being drawn inside and mixed, and the solution can be rapidly and easily dispensed and filtered in one step. Robotic handling of the evidence prevents sample mishandling errors, reduces time for analysis, improves the integrity of the evidence by preventing contamination, and secures the chain-of-custody.

The SC-Tips offer the highest security and integrity of forensic evidence. If the evidence is placed inside of the SC-Tip during the original evidence collection, then the only direct handling of the evidence occurs at collection and during the chemical sample processing in the laboratory. The SC-Tips may be sealed with evidence tape after collection and then submitted to the forensic laboratory for analysis. The SC-Tips may contain bar code labels that uniquely identify the SC-Tip and its contents, either added during evidence collection or subsequently during evidence submission at the forensic laboratory. Further, the SC-Tips may be pre-weighed prior to collection in order to be subsequently weighed in order to make quantitative chemical analyses.

The following examples are intended to illustrate, and not limit, the invention disclosed herein.

EXAMPLES

Example 1

A comparison of recoveries of organophosphates (common pesticides, namely prometon, chlorothalonil and chlorpyrifos) extracted from blended lettuce extract using DPX and HP-DPX is shown in FIG. 7. The peaks in the GC chromatogram obtained following the use of HP-DPX (A) are much higher than those obtained by DPX (B). The higher recoveries are a result of the greater surface area of the smaller particle size material. It should be noted that these extractions were obtained by elution from the top using a 3-way valve adaptor.

Example 2

An example of DPX-CX for the extraction of basic drugs from urine is shown in FIG. 8. The urine was spiked with a mixture of basic drugs at 0.5 ppm, with 0.2 mL extracted, and the resulting chromatogram is shown. All of the basic drugs were rapidly extracted in under 2 minutes and efficiently recovered (>90%) using the DPX-CX tips. The drugs analyzed were methadone (1), methaqualone (2), amitriptyline (3), cocaine (4), imipramine (5), doxepin (6), desipramine (7), SKF (an internal standard (8)), codeine (9) and oxycodone (10). With these tips, one method can be used to effectively extract and analyze practically any basic drug of interest. This example represents the fastest and most efficient method for extracting basic drugs from biological matrices.

Example 3

An example of DPX-IA is exemplified by the HPLC chromatogram depicted in FIG. 9. HPLC chromatogram (with fluorescence detection) was recorded following DPX-IA extraction of 20 ppb aflatoxin B.sub.1 (peak at 6.4 min) from corn meal. Aflatoxin B.sub.1 is a highly toxic substance that is routinely screened in foods such as corn and corn based products. This extraction represents the most selective and fastest of its kind, taking less than 90 seconds to perform (following the blending of the sample). The addition of methanol (or acetone or other solvent) causes the antibody to denature and thus "release" the bound analyte. Due to the high selective binding of antibodies, negligible interference from sample matrix components occurs, even given the low concentration of analyte. The extraction, after blending the sample matrix with solvent, took much less time than the chromatographic analysis (with the peak at 6.4 min (arrow) being aflatoxin B.sub.1.

Example 4

In FIG. 10, GC/MS chromatograms of DPX extracts of stomach contents is shown with and without the use of DPX-CU. Without DPX-CU (B), the chromatogram is characterized with intense peaks associated with sample matrix compounds predominantly consisting of fatty acids. These peaks interfere with the analysis of potentially co-eluting peaks of analytes of interest. The chromatogram obtained following the use of DPX-CU (A) demonstrates no interfering peaks, even with the scale of the y-axis at approximately 50 times less (4.5.times.10.sup.6 in A compared to 1.1.times.10.sup.8 in B).

Example 5

In FIG. 11A, 1 mL of whole blood is collected by a forensic pathologist and injected onto the top of the SC-Tip 92 that is bar code labeled and contains absorbent material and a screen 94. The cap 91, which also acts as a transport adaptor, is securely placed on the SC-Tip and evidence tape is placed over it, and his initials and time are subsequently written on the tape and evidence submission form (with corresponding bar code label). The adaptor and top of the pipette tip may be threaded to be used as a screw cap. A small plug or cap at the bottom (narrow end) of the tip may also be used to provide a complete seal of the DPX-collection tip. The sample may also be a cotton swab (B), or similar material for collecting samples. The evidence is shipped to the laboratory for testing. Once the evidence is received, the SC-Tip is immediately scanned with a bar code scanner and placed in a rack to be processed robotically without any manual transferring of the evidence. The chain-of-custody begins with the sample collection (pathologist) and ends with the chemical analysis without any other "links" in the chain. The analysis is initiated almost as soon as the sample is received with little to no downtime in order to facilitate case turnaround time.

Example 6

A law enforcement officer makes a routine traffic stop and notices a bag of suspect powder material. He places a small spatula full of this sample into a SC-Tip (bar code labeled), places the cap or adaptor onto the tip, attaches evidence tape, and writes his initials and time/date onto the evidence tape and corresponding evidence submission form. (He also maintains the rest of the evidence to be weighed and re-analyzed if necessary). The evidence is shipped to the laboratory, and the SC-Tip is immediately processed robotically without any delays. The analysis is initiated before the evidence paper work is submitted into the computer or laboratory information management system.

Example 7

In FIG. 11B, a swab of sample is contained inside of the SC-Tip. The swab may be directly processed using robotics for the analysis of 1) DNA collected from suspects (or criminals); 2) drugs from oral fluid collected; and 3) gunshot residue particles from hands of firearm suspects. The robotics may be coupled to capillary electrophoresis, GC-MS or HPLC-MS/MS, or ICP or ICP/MS instrumentation for the analysis of DNA, drugs or explosives, or gunshot residue particles, respectively.

Those with ordinary skill in this area will recognize that the invention is not limited to the specific embodiments described above, but it also includes variations that are equivalent to the invention disclosed herein.

* * * * *

US 20150011016A1

(19) United States

(12) Patent Application Publication

(10) Pub. No.: US 2015/0011016 A1

(43) Pub. Date: Jan. 8, 2015

Brewer

(54) DISPERSIVE PIPETTE EXTRACTION TIP AND METHODS FOR USE

(71) Applicant: **William BREWER**, Columbia, SC (US)

(72) Inventor: **William E. Brewer**, 26 Cedar Field Court, SC (US)

(21) Appl. No.: **14/379,227**

(22) PCT Filed: **Feb. 14, 2013**

(86) PCT No.: **PCT/US2013/026217**

§ 371 (c)(1),
(2) Date: **Aug. 15, 2014**

Related U.S. Application Data

(60) Provisional application No. 61/599,057, filed on Feb. 15, 2012.

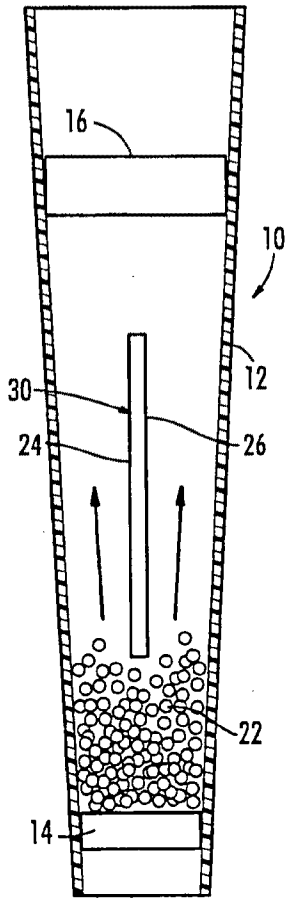
Publication Classification

(51) **Int. Cl.**
G01N 1/40 (2006.01)
B01L 3/02 (2006.01)

(52) **U.S. Cl.**
CPC ***G01N 1/405*** (2013.01); ***B01L 3/0275*** (2013.01); *B01L 2200/0631* (2013.01); *B01L 2300/0681* (2013.01); *B01L 2400/086* (2013.01)
USPC **436/501**; 422/514; 422/70; 422/68.1; 436/178; 436/68

(57) **ABSTRACT**

A pipette tip device for use in dispersive SPE. The device includes a pipette tip having a lower barrier, loose sorbent that is freely moveable during the extraction process, and a baffle system that is shaped to disrupt the flow of liquid sample that is aspirated into the pipette tip. The baffle system includes an insert that may be separate from or monolithic with the interior of the pipette tip.



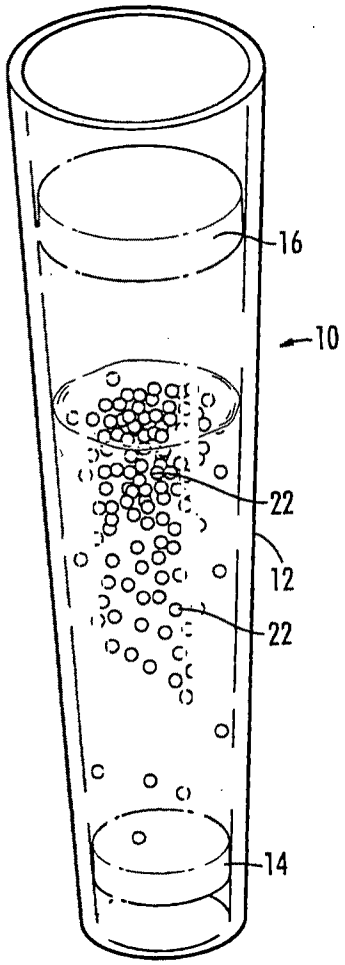


FIG. 1
PRIOR ART

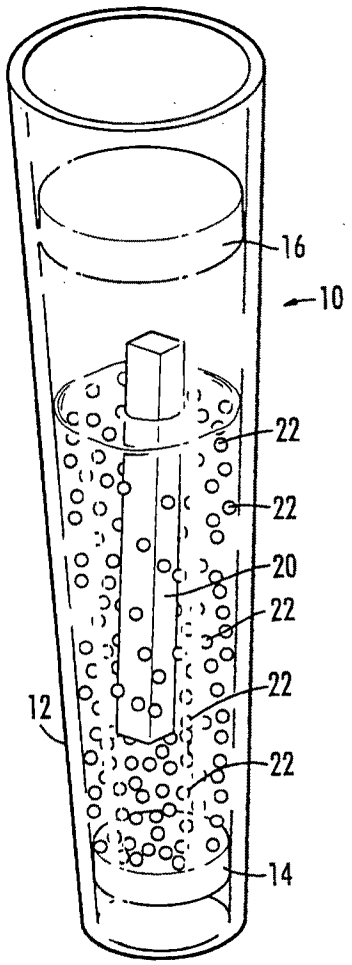


FIG. 2

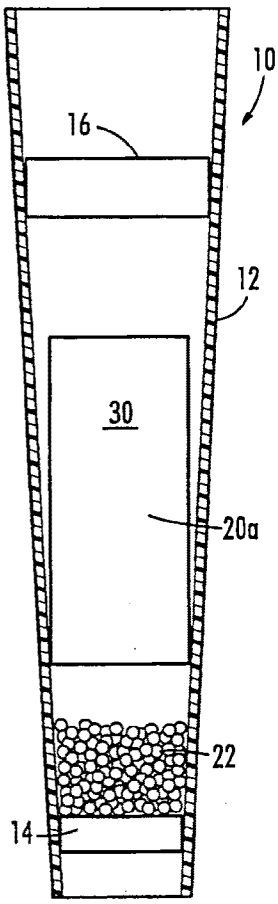


FIG.3A

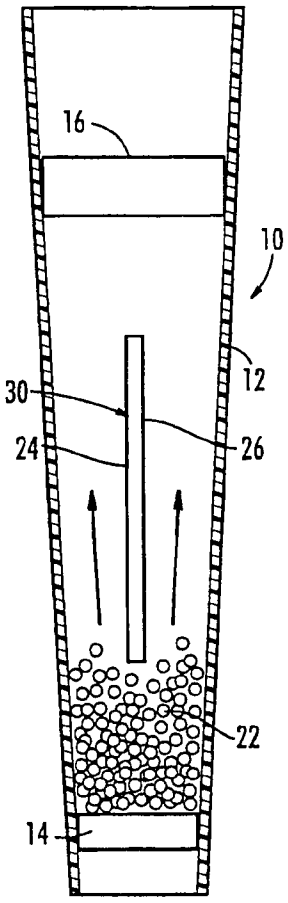


FIG.3B

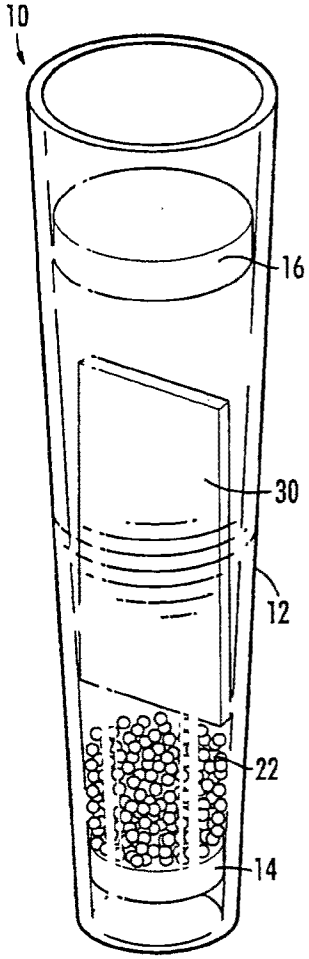


FIG.3C

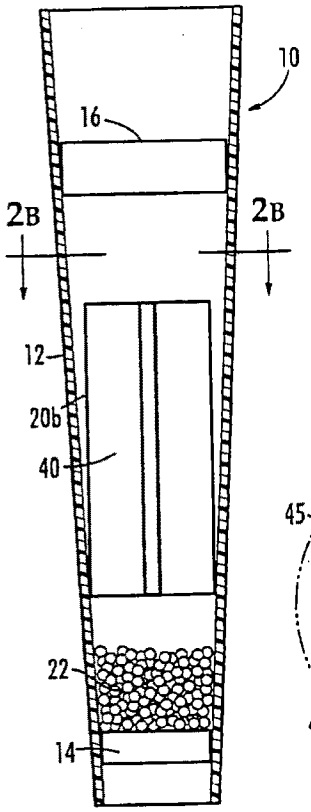


FIG. 4A

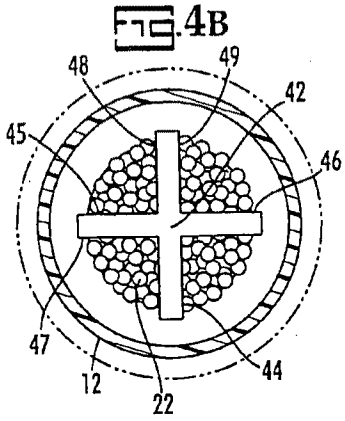


FIG. 4B

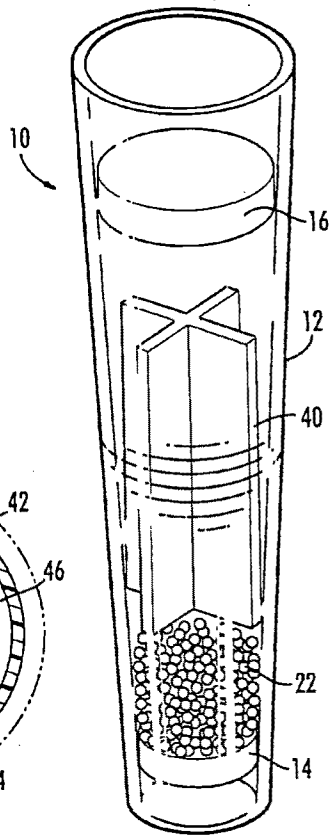


FIG. 4C

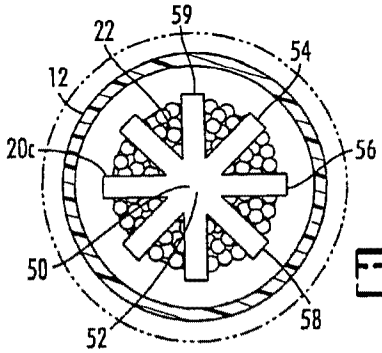


FIG. 4D

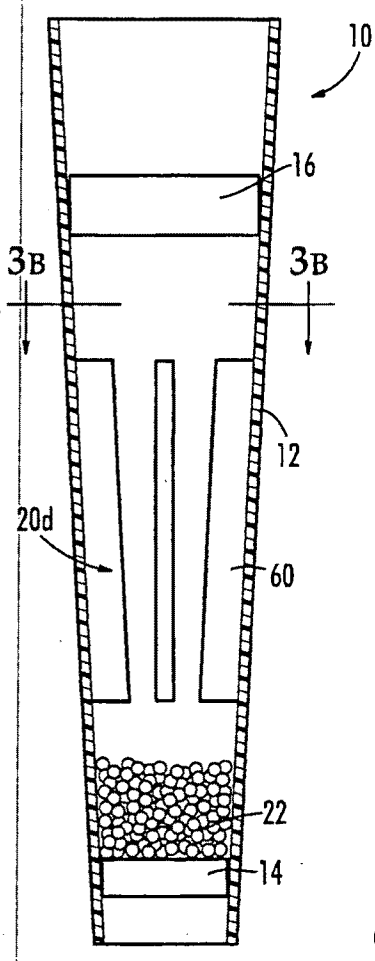


FIG.5A

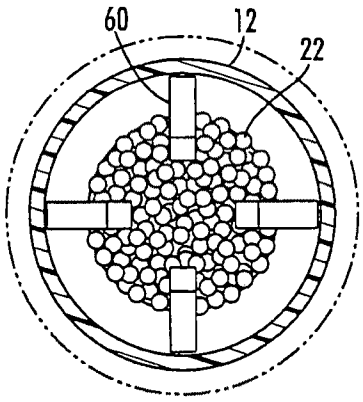


FIG.5B

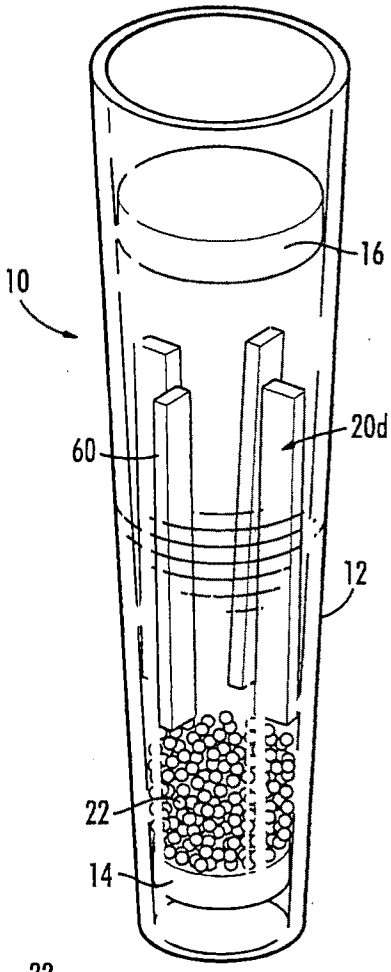


FIG.5C

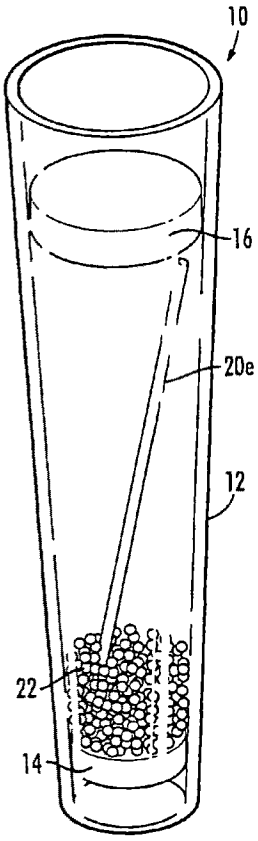


FIG.6

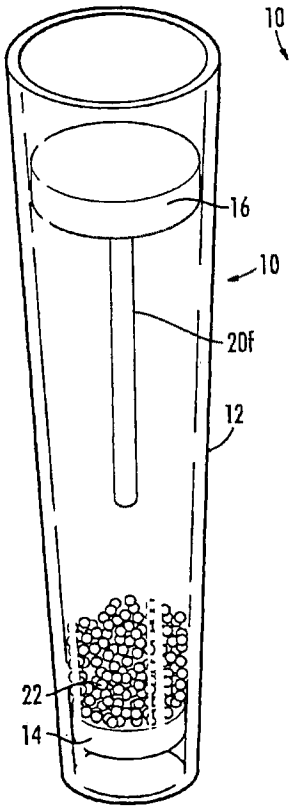


FIG.7

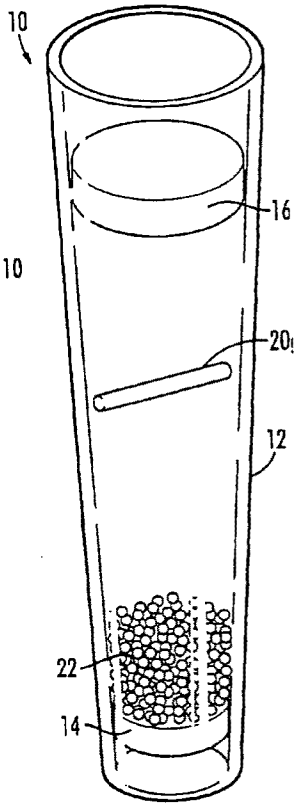


FIG.8

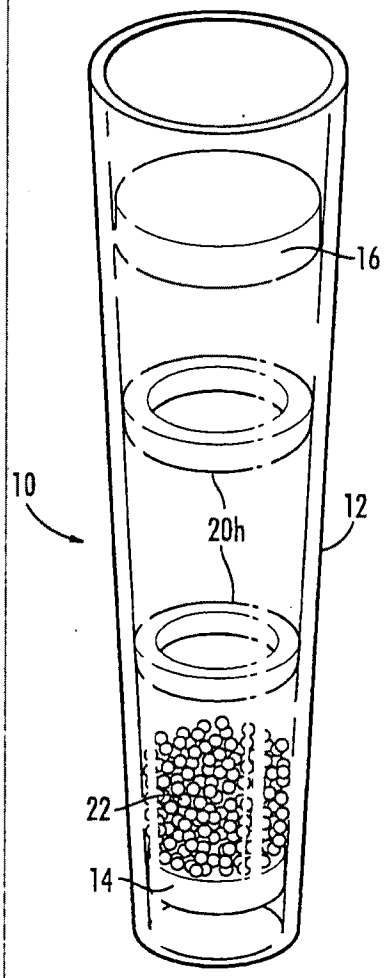


FIG.9

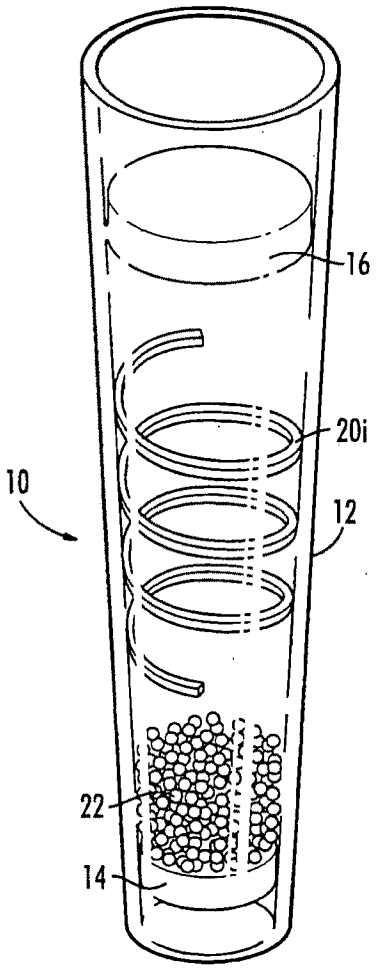


FIG.10

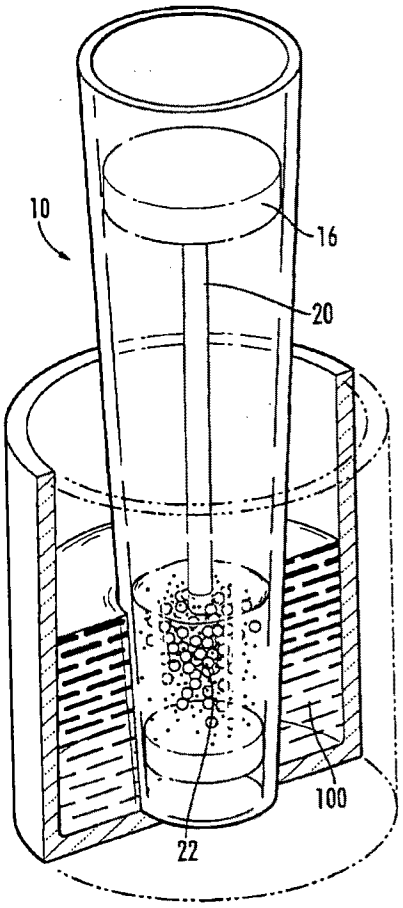


FIG. 11A

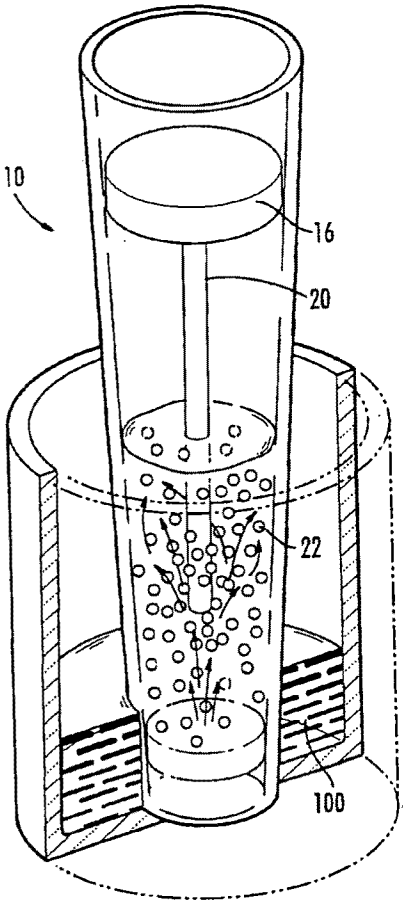


FIG. 11B

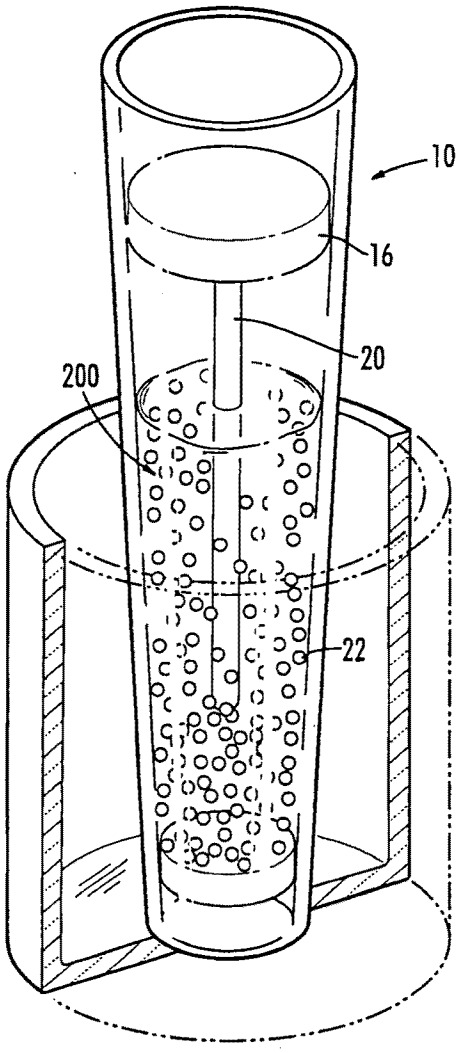


FIG.11C

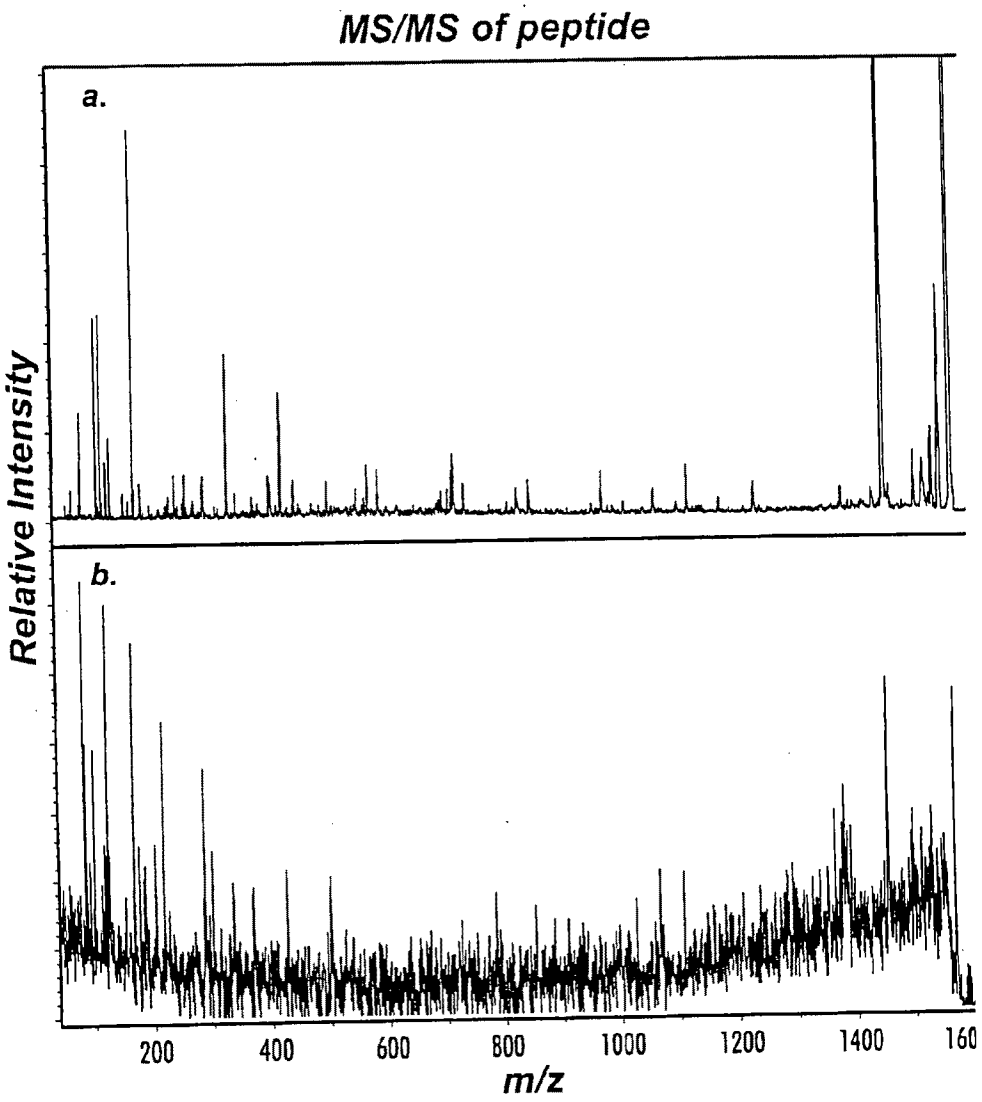

MS/MS of peptide
a.
b.
Relative Intensity
200
400
600
800
1000
1200
1400
160
m/z

FIG. 12

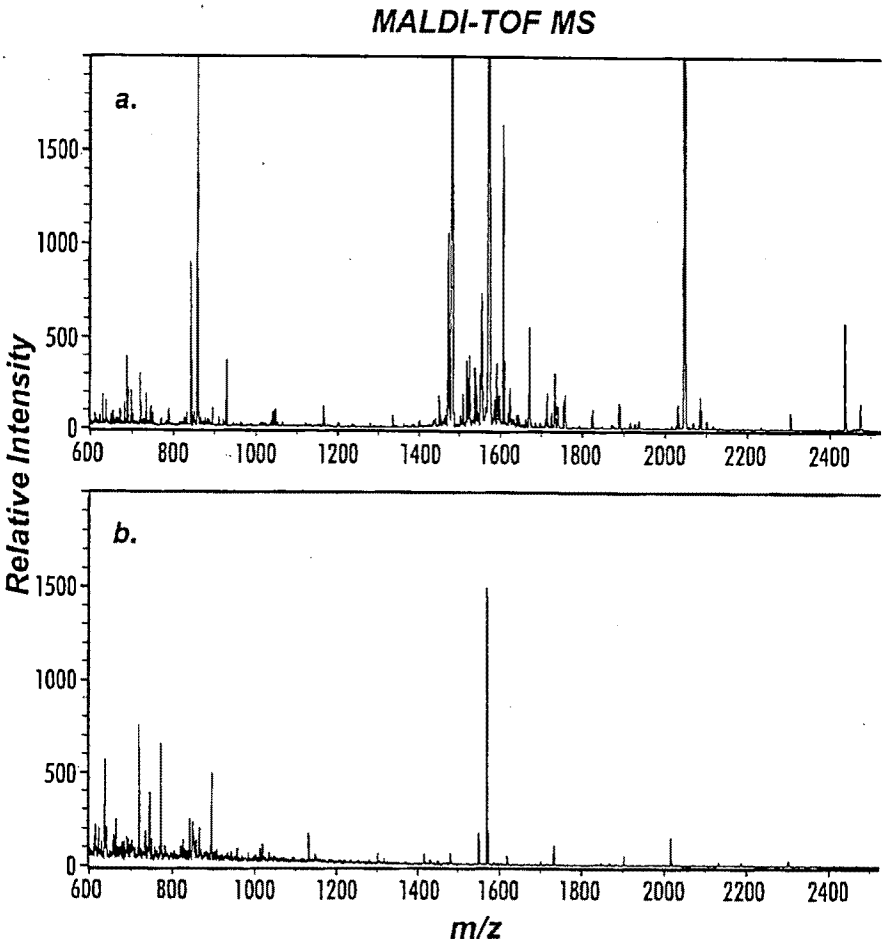

MALDI-TOF MS
a.
b.
Relative Intensity
m/z
0
500
1000
1500
600
800
1000
1200
1400
1600
1800
2000
2200
2400

FIG.13

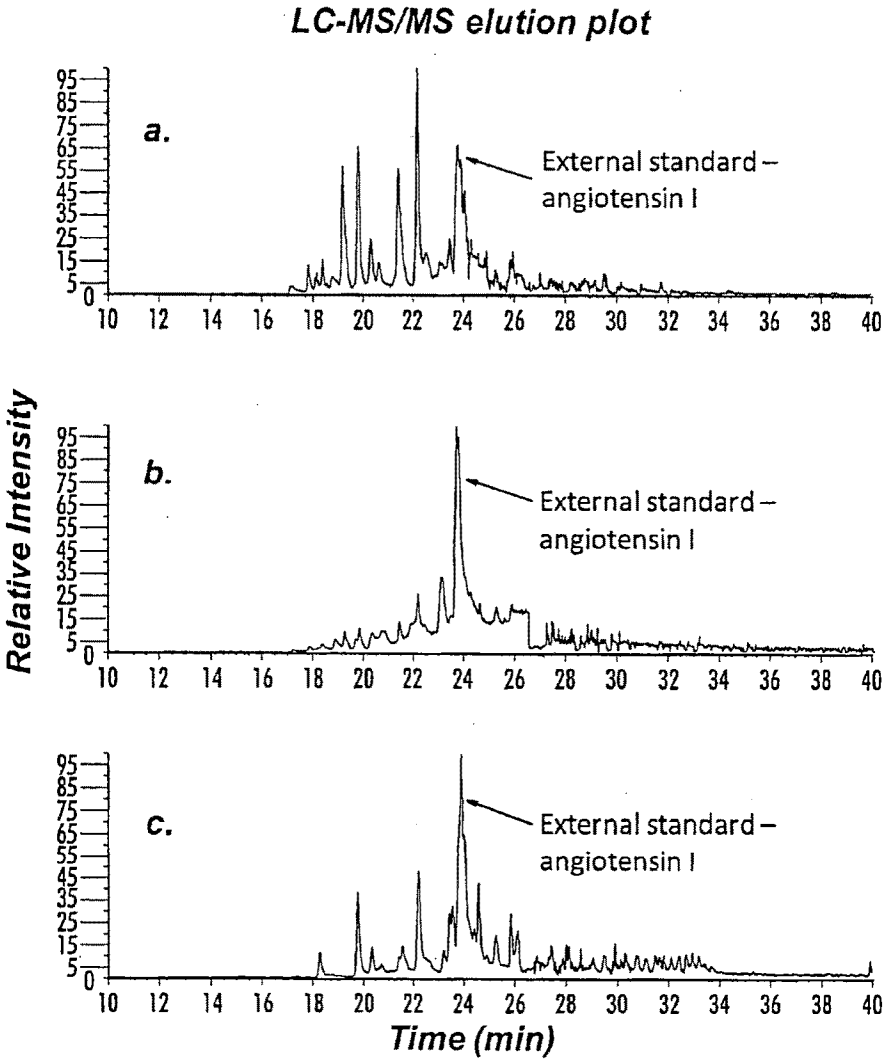

LC-MS/MS elution plot
a.
b.
c.
External standard – angiotensin I
Relative Intensity
Time (min)
95
85
75
65
55
45
35
25
15
5
0
10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40

FIG. 14

DISPERSIVE PIPETTE EXTRACTION TIP AND METHODS FOR USE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of priority of U.S. Provisional Application No. 61/599,057, filed on Feb. 15, 2012, which is incorporated herein in its entirety.

FIELD OF THE INVENTION

[0002] The present invention relates generally to an extraction device used during sample preparation for chemical analysis.

BACKGROUND OF THE INVENTION

[0003] Pipette tips for solid phase extraction (SPE) have been developed. Many of these use immobilized sorbent at the narrow end of the tip. The problems with these types of tips include solvent flow rates have to be controlled and the efficiency of the sorbent is not optimal because solution contact with the surface area of the sorbent is limited in fixed beds.

[0004] Some pipette extraction products, such as disposable pipette extraction products, including those described in U.S. Pat. No. 6,566,145, use loosely contained sorbent inside of the pipette tip. The sorbent is contained through the use of a screen or filter and an upper porous barrier. Being loosely contained, the sorbent is freely movable and is mixed with solutions providing unsurpassed efficiencies. Additionally, the extractions are rapid as compared to typical extraction methods because conditioning steps and slow flow rates are not required. This type of pipette tip extraction device utilizes dispersive SPE. As used herein, "dispersive" means: the solid phase sorbent may be thoroughly mixed with liquid solutions aspirated into the pipette tip.

[0005] While suitable for larger pipette tips, the disposable pipette extraction does not work well with narrow and low volume tips. For example, 5 mL and 1.3 mL disposable pipette extraction tips work well for extracting drugs from urine or pesticides from fruit and vegetable extracts. However, the disposable pipette extraction method is irreproducible when incorporated in 1 mL pipette tips that are used for 96 well plates and robotics. The poor reproducibility is mainly a result of inefficient mixing of the sorbent material with the sample solution. The sorbent bed after conditioning tends to adhere to the walls of the narrow tip, and consistent mixing with the sample solution is therefore difficult to achieve. Oftentimes, the sorbent bed floats on top of the sample solution rather than interacting with the solution, and irreproducibility occurs.

[0006] To overcome this problem, one device, known commercially by the name ASPIRE®, incorporates an intermediate porous barrier. The intermediate porous barrier ensures the sorbent is mixed with the solutions by aspirating and dispensing the solutions through the intermediate barrier. The intermediate barrier allows liquid solutions to pass through, but prevents the sorbent from passing through. However, a drawback to this type of extraction tip is that the intermediate barrier may cause losses in recovery because some of the liquid solution will inevitably get trapped by the porous membrane. Another drawback is that the existence of this barrier creates back pressure issues.

[0007] Accordingly, there exists a need for a dispersive SPE device that may be used to process liquid sample solutions for reproducible chemical analysis with low back pressure.

SUMMARY OF THE INVENTION

[0008] The following presents a simplified summary of the invention in order to provide a basic understanding of some aspects of the invention. This summary is not an extensive overview of the invention. It is not intended to identify key or critical elements of the invention or to delineate the scope of the invention; its sole purpose is to present concepts of the invention in a simplified form as a prelude to the more detailed description that is subsequently presented.

[0009] The present invention provides a pipette tip device for the extraction of liquid sample solutions. The device includes a pipette tip having a lower barrier, such as a frit, or screen at the narrow or lower end, and an upper barrier that is porous at the wide opening or upper end of the tip. Between these barriers is contained loose sorbent that is freely moveable during the extraction process.

[0010] Additionally, the device includes a baffle system interior to the pipette tip to facilitate dispersion and mixing by the development of turbulence as liquid is aspirated. Importantly, the baffle system causes the sorbent and the liquid sample to mix turbulently. The baffle system may comprise a dispersive insert, including a single piece presenting multiple faces to the fluid, each at an angle with respect to an adjacent surface or plural spaced-apart inserts with multiple, angled faces. The insert or inserts may be integral with or separate from the interior of the pipette tip. Furthermore, the insert or inserts may be movable or fixed within the pipette tip.

[0011] The flat surfaces parallel to fluid movement encourage mixing as a result of laminar flow; the angles at which adjacent surfaces are set produces mixing by turbulence. The baffle system may be located between the upper barrier and the lower barrier and may generally be parallel to or at an angle with the cylindrical axis of the pipette. The present baffle system also disrupts annular flow, but does not significantly retard axial flow as fluids are drawn in and expelled out. As used herein, the term "disrupt" means to introduce cross-flows, counter-flows or turbulence, and combinations thereof in flow rate of the fluid with respect to the sorbent in order to promote mixing time between aspiration and expulsion of the fluid from the pipette tip containing sorbent.

[0012] By way of example, the insert may be a single piece that is located between the upper and lower barriers, and above the sorbent bed. The insert is capable of disrupting or is shaped to disrupt the sorbent bed, and causing the sorbent to disperse into the liquid solution. As used herein, "shaped to disrupt" refers to a structure that is of sufficient size with respect to the pipette's internal dimensions to disrupt the fluid or sorbent movement during the aspiration of fluid into a pipette tip containing sorbent.

[0013] Normally, when liquid solutions are aspirated into the pipette extraction tip, the sorbent will tend to float and move upward into the tip and to the top of the liquid surface. Furthermore, the sorbent can form clumps. The insert disrupts this flow and prevents potential clumping. Not only does the insert act as a physical barrier that pushes and forces the sorbent to disperse and mix with the solution being aspirated, the insert disrupts the flow of the liquid sample by causing turbulence, which promotes the mixing between the sorbent and the liquid.

[0014] The insert may be a separate part that is introduced into the pipette tip device. Alternatively, the insert may be integral with or monolithic with the interior of the pipette tip. The insert may be nonporous or substantially nonporous so that the flow of liquid moves around the insert rather than through it. A smoother or flatter surface facilitates the liquid flow around the insert, which in turn creates additional turbulence to facilitate mixing. Should liquid flow through a porous insert, back pressure issues may result, as well as losses in recoveries.

[0015] There are various designs of inserts that may be utilized. One insert may be flat and rectangular or square in shape that is inserted into the pipette tip. Such insert provides two opposing faces for use in the dispersing of the sorbent through turbulent mixing with the liquid sample. As used herein, "turbulent mixing" refers to the combining of a liquid with a sorbent material in a tumultuous or disorderly manner.

[0016] Another example includes the use of a rod-like or wire-like, round insert that extends along the length of the pipette tip, about parallel to the cylindrical axis and into the narrow end of the tip. Alternatively, a wire-like insert could extend across the width, perpendicular to or at an angle with, the cylindrical axis of the pipette tip. In still another alternative, the rod-like insert could be connected to the upper barrier. These embodiments also facilitate turbulent mixing between the sorbent material and the liquid sample by disrupting the flow of the liquid and the sorbent movement.

[0017] Another insert may include a central stem having one or more ribs extending radially out from the cylindrical axis of the pipette tip, which provide multiple opposing faces for use in dispersing the sorbent. For example, the insert may include intersecting ribs, such as two rib members that perpendicularly intersect along the axis of the central stem to provide a cross-like shape from a top view. Alternatively, multiple intersecting ribs may be employed to provide additional shapes that form separate wedge-like spaces between the ribs. As with the prior embodiments, these provide sufficient disruption of the sorbent material by facilitating turbulent mixing.

[0018] Yet another insert may include one or more ridges or protrusions along the interior surface of the pipette tip. These ridges may be linear, and substantially parallel to or at an angle with the cylindrical axis of the pipette tip, and may extend radially toward the cylindrical axis of the pipette tip. Alternatively, these ridges may be curved, or spiral-like in design, and may extend radially, longitudinally and azimuthally with respect to the cylindrical axis of the pipette tip. Again, these embodiments provide for turbulent mixing between the sorbent material and the liquid sample.

[0019] Another feature of the insert is that it may be formed to serve a mechanical and/or chemical function during the extraction or chemical analysis. For example, the insert may have a surface with reactive properties or agents that may affect either the extraction of the analyte or the sample matrix, or have the ability to perform an immunoassay screen or measure pH. If only serving a mechanical function, the surface of the insert or inserts may be flat, nonporous and inert. Thus, the pipette tip device of the present invention could be used not only to extract drugs from a sample, such as a urine sample, but it could also be employed to simultaneously perform an immunoassay drug screen or test for adulterants.

[0020] Other features and their advantages will be readily apparent to those skilled in chemical arts from a careful reading of the Detailed Description of Preferred Embodiments, accompanied by the following drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] FIG. 1 includes a perspective view of a prior pipette tip device having no baffle system;

[0022] FIG. 2 includes a perspective view of a pipette tip device having a baffle system according to an embodiment of the present invention;

[0023] FIG. 3A includes a cross sectional view of a pipette tip device showing the front of a baffle system according to an embodiment of the present invention;

[0024] FIG. 3B includes a cross sectional view of a pipette tip device showing the side of a baffle system according to an embodiment of the present invention;

[0025] FIG. 3C includes a perspective view of a pipette tip device according to an embodiment of the present invention;

[0026] FIG. 4A includes a cross sectional view of a pipette tip device showing the front of a baffle system according to an alternative embodiment of the present invention;

[0027] FIG. 4B includes a cross sectional view of a pipette tip device at cross section line 2B according to an alternative embodiment of the present invention;

[0028] FIG. 4C includes a perspective view of a pipette tip device according to an alternative embodiment of the present invention;

[0029] FIG. 4D includes a cross sectional view of a pipette tip device showing the top of a baffle system according to an alternative embodiment of the present invention;

[0030] FIG. 5A is a cross sectional view of a pipette tip device according to an alternative embodiment of the present invention;

[0031] FIG. 5B is a cross sectional view of a pipette tip device at cross section line 3B according to an alternative embodiment of the present invention; and

[0032] FIG. 5C is a perspective view of a pipette tip device according to an alternative embodiment of the present invention.

[0033] FIG. 6 is a perspective view of a pipette tip device according to an alternative embodiment of the present invention;

[0034] FIG. 7 is a perspective view of a pipette tip device according to an alternative embodiment of the present invention;

[0035] FIG. 8 is a perspective view of a pipette tip device according to an alternative embodiment of the present invention;

[0036] FIG. 9 is a perspective view of a pipette tip device according to an alternative embodiment of the present invention;

[0037] FIG. 10 is a perspective view of a pipette tip device according to an alternative embodiment of the present invention;

[0038] FIG. 11A is a perspective view of a pipette tip device being used in an extraction step according to a method of the present invention;

[0039] FIG. 11B is a perspective view of a pipette tip device being used in an extraction step according to a method of the present invention;

[0040] FIG. 11C is a perspective view of a pipette tip device being used in an extraction step according to a method of the present invention;

[0041] FIG. **12** is a chromatogram comparing dispersive pipette tip technology to fixed resin pipette tip technology according to an embodiment of the present invention;

[0042] FIG. **13** is a chromatogram comparing dispersive technology to fixed resin pipette tip technology according to an embodiment of the present invention; and

[0043] FIG. **14** is a chromatogram comparing dispersive pipette tip technology compared to fixed resin pipette tip technology according to an embodiment of the present invention.

DETAILED DESCRIPTION

[0044] The present invention provides a pipette tip device **10** and method for the extraction of liquid sample solutions. The device includes a pipette tip **12** having a lower barrier **14**, such as a frit, or screen a closure with a slit, or a closure with multiple slits, at the narrow or lower end, and an upper barrier **16** that is porous at the wide opening or upper end of the tip. Between these barriers is contained loose sorbent **22** that is freely moveable during the extraction process.

[0045] Additionally, the device **10** includes a baffle system **20** interior to the pipette tip **12** to facilitate dispersion and mixing between the sorbent **22** and the liquid sample by the development of turbulence when the pipette tip **12** is aspirated with liquid. The use of the baffle system **20** promotes enhanced and consistent results when performing solid phase extractions.

[0046] As shown in FIG. **1**, prior pipette tip devices that do not include a baffle system **20** tend to result in clumping of the sorbent **22** and ultimately in mixtures that are varied and inhomogenous. Thus, the extractions are not entirely effective or reproducible. With the inclusion of a baffle system **20**, such as shown in FIG. **2**, however, a homogenous or uniform mixture results when the sorbent **22** and the liquid sample are combined.

[0047] The shape and dimension of the baffle system **20** can vary provided that it facilitates or creates turbulent mixing between the sorbent material **22** and a liquid sample. Particularly, the baffle system **20** functions to disrupt the flow of the liquid sample and sorbent movement through the pipette tip **12** by causing turbulence and mixing.

[0048] The surface of the baffle system **20** may be flat or smooth, nonporous, or substantially nonporous and inert. Alternatively, the surface may include reactive properties. For example, the baffle system **20** may have a surface with reactive properties or agents that may affect either the extraction of the analyte or the sample matrix, or have the ability to perform an immunoassay screen or measure pH.

[0049] In one embodiment, shown in FIGS. **3**A-3C, the baffle system **20***a* is between the upper and lower barriers, **16**, **14**, respectively, and above the sorbent **22**. In this embodiment, the baffle system **20***a* includes a single insert **30** that is generally flat and rectangular in shape with a first face **24** opposing a second face **26**.

[0050] In this embodiment, the insert **30** is dimensioned to be inserted into the wide, upper end of the pipette tip **12**. Particularly, the shape of the insert is such that the base **32** of the insert **30** becomes lodged or abuts the interior walls of the narrow, lower end of the pipette tip **12** at either side of the lower end of the insert **30**. As shown, the insert **30** may be placed upright so that it is about parallel to the cylindrical axis of the pipette tip **12**. Alternatively, the insert **30** may be placed at a lean and at an angle with the cylindrical axis of the pipette tip **12**. Moreover, the insert **30** may be moveable or fixed within the pipette tip **12** when liquid sample is introduced.

[0051] As shown in FIG. **1***b*, baffle system **20***a* serves to disrupt liquid flow by forcing the liquid and sorbent to move around the insert **30**, and thereby enhance the uniform mixing of the sorbent material **22** as liquid is aspirated into the pipette tip **12**. Generally, the properties of the baffle system **20***a* are such that turbulent mixing between the liquid sample and the sorbent material **22** is facilitated, and sorbent clumping or non-uniform mixing is avoided.

[0052] Particularly, the bottom of the insert **30** provides a physical barrier to the upward movement of the sorbent and liquid. Based on the shape and location of the insert, the sorbent and liquid must move around it and flow next to the opposing faces **24** and **26**. By forcing the sorbent and liquid to move to either side of the insert **30**, additional turbulence is created. Furthermore, the opposing faces **24** and **26** may be at an angle with respect to the adjacent walls of the pipette tip **12**, which further disrupts the flow of the liquid and movement of the sorbent **22**.

[0053] An alternative embodiment of the baffle system is shown in FIGS. **4**A-4C. Particularly, the baffle system **20***b* includes an insert **40** with a stem **42** that is an axially-extending construction between the upper and lower barriers **16** and **14**. The stem **42** is formed by two axially-extending rib members, **44** and **46**. The rib members **44** and **46** perpendicularly intersect along the axis of the stem **42**. Further, the rib members each provide multiple, opposing faces, **45**, **47** and **48**, **49**.

[0054] As shown, the insert **40** may be placed upright within the pipette tip **12** so that the rib members **44** and **46**, extend out radially from the axis of the stem **42**, which is about co-axial with the cylindrical axis of the pipette tip **12**. Alternatively, the insert **40** may be placed at an angle other than about zero with respect to the cylindrical axis of the pipette tip **12**.

[0055] As with the prior embodiment, the insert **40** of baffle system **20***b* is shaped to disrupt the sorbent material **22** and a liquid sample. In use, the baffle system **20***b* forces the flow of the liquid sample and sorbent **22** around the insert **40**, which creates turbulence for enhanced mixing between the sorbent and the liquid.

[0056] It is therefore contemplated by the present invention that the baffle system **20** may include a variety of shapes and dimensions to disrupt sorbent **22** and liquid samples. For example, the baffle system **20***c*, shown in FIG. **4**D is similar to the baffle system **20***b* except that additional rib members are included. In particular, the baffle system **20***c* includes an insert **50** having a stem **52** formed by four intersecting rib members **54**, **56**, **58** and **59**. The ribs are shown as evenly spaced apart. Alternatively, the ribs may be unevenly spaced. Other baffle systems of this type may include one or more ribs that extend out from a stem.

[0057] Another alternative embodiment is shown in FIGS. **5**A-5C. In this embodiment, the baffle system **20***d* includes one or more ridges **60** or protrusions that extend out from the interior surface of the pipette tip **12**. As illustrated, each ridge **60** may be linear in shape and extend vertically along the length of the pipette tip **12** from the upper end to the lower end of the tip **12**, and between the upper and lower barriers, **16** and **14**. Particularly, the ridges **60** may be spaced apart evenly. Alternatively, the ridges **60** are spaced apart at uneven intervals. Importantly, the ridges **60** disrupt the liquid sample by

promoting turbulent mixing and by creating additional turbulence in the flow of the liquid sample and the movement of the sorbent **22**.

[0058] In yet another alternative embodiment, wire-like or rod-like baffle systems that are rounded in shape are used. In one example, shown in FIG. **6**, a rod-like baffle system **20***e* is shown between the upper and lower barriers **16** and **14**, and placed at a lean with respect to the cylindrical axis of the pipette tip **12**. Alternatively, in FIG. **7**, a rod-like baffle system **20***f* is shown as being connected with the upper barrier **16** and extending vertically down into the pipette tip **12**. Still another example, shown in FIG. **5**, includes a rod-like baffle system **20***f* that extends horizontally across the interior of the pipette tip **12**.

[0059] Even other alternative embodiments are shown in FIGS. **9-10**. The baffle system **20***h* in FIG. **9** includes ridges along the interior of the pipette tip **12** that may extend horizontally between the upper and lower barriers, **16** and **14**. Such ridges may be annular in shape. Alternatively, the baffle system **20***i* includes ridges along the interior of the pipette tip **12** that may have a spiral shape and that may run clockwise or counter-clockwise, as shown in FIG. **10**.

[0060] Among the alternative types of baffle systems, each includes surfaces that participate in the disruption of the sorbent **22** during the extraction process. Such surfaces may have a variety of properties. In some instances, the surfaces of the baffle system may be flat or smooth, inert and nonporous. In other instances, the surfaces of the baffle system may be rounded or generally not uniform. Furthermore, reactive properties may be included on the surfaces. Moreover, among the alternative types, each of the baffle system may either be separate from or integral and monolithic with the pipette tip. Thus, each baffle system is shaped to disrupt to the liquid sample by promoting turbulent mixing between the sorbent **22** and the liquid sample.

[0061] The present invention further contemplates a method of dispersive SPE through the use of the pipette tip device **10**. In one embodiment of the method of the present invention, various steps are shown in FIGS. **11A-11C**. Particularly, the method may include the following steps: 1) providing a dispersive SPE pipette tip device **10**, wherein the pipette tip includes a baffle system **20** as described above; 2) providing a sample solution **100**; 3) aspirating the sample solution **100** into the pipette tip device **10**; and 4) combining the sorbent **22** with the sample **100** in the presence of the baffle system **20** to form a uniform mixture **200** and to perform the SPE extraction. Additionally, the method may include the steps of collecting the extracts, or, alternatively, dispensing the resulting solution to waste, and then repeating the steps 3) and 4) with wash and elution solvent.

[0062] As illustrated in FIG. **11A**, when the liquid sample **100** is first drawn into the pipette tip device **10**, the sorbent submerges and absorbs the liquid. The baffle system **20**, in FIG. **11B** next disrupts the liquid sample **100** as it continues to be aspirated into the pipette tip. In turn, the disruption creates a uniform mixture **200** of the sorbent **22** and the liquid **100**, as shown in FIG. **11C**. This method and device, therefore, enhances the effectiveness and reproducibility of SPE extractions.

[0063] The following examples are intended to illustrate, and not limit, the invention disclosed herein.

EXAMPLES

Example 1

[0064] A drug mixture of opiates is extracted from urine using the dispersive SPE pipette tip device **10**. The first series of extractions (a) use pipette tips without a baffle system, and the second series (b) of extractions use pipette tips with a baffle system. The final extracts were analyzed by liquid chromatography with tandem mass spectrometry (LC/MS/MS). The results of 8 replicates of the pipette tips without baffle systems show relative peak intensities with 15.6% relative standard deviation (RSD). However, with the baffle system in place, the % RSD is less than 5%. In fact, the % RSD of the peak intensities with the baffle system are relatively identical to the % RSD of the peak intensities of a neat (unextracted) standard of opiates injected repeatedly into the LC/MS instrument.

Example 2

[0065] As shown in FIG. **12**, a MALDI-TOF MS of trypsin digested bovine serum albumin (BSA) (1 μg) after reverse phase extraction with two different micropipette tips. The results for (a.) were taken after a dispersive pipette tip device **10** of the present invention was aspirated two times with a liquid sample. The results for (b.) were taken after a fixed resin reverse phase pipette tip was aspirated ten times with a liquid sample. Comparing (a.) including the use of pipette tip device **10** of the present invention (dispersive technology) to (b.) including the use of fixed resin reverse phase pipette tips, the results show higher signal intensities with greater sequence coverage for samples recovered using dispersive pipette tips **10**. The relative intensity for (a.) has been normalized to (b.) for comparison purpose.

Example 3

[0066] As shown in FIG. **13**, tandem mass spectrometry of a peptide (m/z 1568) obtained on MALDI-TOF/TOF. The results for (a.) were taken after a dispersive pipette tip device **10** of the present invention was aspirated two times with a liquid sample. The results for (b.) were taken after a fixed resin reverse phase pipette tip was aspirated ten times with a liquid sample. Comparing (a.) dispersive technology to (b.) fixed resin reverse phase pipette tips, the peptide fragmentation signals have higher signal to noise ratios with full coverage of all y and b ions for (a.). The two spectra are not to scale with respect to each plot.

Example 4

[0067] As shown in FIG. **14**, LC elution plots against MS intensities obtained from LTQ-Orbitrap Velos Pro of trypsin digested BSA de-salted and concentrated using dispersive micropipette tips (a. and c.) compared to fixed resin tips (b.). A stock of trypsin-digested BSA solution was aliquoted (3 pmol in 50 μL) and de-salted using dispersive technology (a.) and fixed resin (b.) then eluted with acetonitrile, followed by solvent evaporation and re-suspension in 10 μL of 2% acetonitrile in 0.1% formic acid/water containing same concentration of angiotensin I, an external standard. One μL of the solution was injected and analyzed. (c.) The stock trypsin-digested BSA solution was further diluted 10-fold (300 femptomol (fmol) in 50 μL), then de-salted/concentrated to 10 μL using dispersive micropipette tip and injected 1 μL for analysis. The results for (a.) and (c.) were taken after a dispersive

pipette tip device **10** of the present invention was aspirated two times with a liquid sample. The results for (b.) were taken after a fixed resin reverse phase pipette tip was aspirated ten times with a liquid sample. Comparing (a.) and (c.) dispersive technology to (b.) fixed resin reverse phase pipette tips, the signals have higher signal to noise ratios and the results show more abundant peaks. This indicates that the dispersive technology in this example provides over a 10-fold improvement in sensitivity.

[0068] Those skilled in the chemical arts will appreciate from the foregoing description of preferred embodiments that substitutions and modification may be made without departing from the spirit and scope of the invention which is defined by the appended claims.

What is claimed is:

1. A pipette tip device for performing dispersive SPE, comprising:

a pipette tip having a lower barrier;

sorbent material contained above said lower barrier; and

a baffle system contained within said pipette tip, wherein said baffle system is shaped to disrupt the movement of said sorbent material when a liquid sample is introduced into said pipette tip.

2. The pipette tip device as recited in claim **1**, wherein said lower barrier is a frit, a screen, a closure with a slit, or a closure with multiple slits.

3. The pipette tip device as recited in claim **1**, further comprising an upper barrier, wherein said sorbent material is between said upper barrier and said lower barrier.

4. The pipette tip device as recited in claim **3**, wherein said baffle system is connected with said upper barrier.

5. The pipette tip device as recited in claim **3**, wherein said upper barrier is a porous frit.

6. The pipette tip device as recited in claim **1**, wherein said baffle system includes an insert that is above said sorbent material.

7. The pipette tip device as recited in claim **6**, wherein said insert is a single, flat piece.

8. The pipette tip device as recited in claim **7**, wherein said piece is rectangular in shape.

9. The pipette tip device as recited in claim **7**, wherein said piece is round or oval.

10. The pipette tip device as recited in claim **7**, wherein said piece is monolithic with the interior surface of said pipette tip.

11. The pipette tip device as recited in claim **1**, wherein said baffle system includes an insert having an elongated cylindrical shape.

12. The pipette tip device as recited in claim **1**, wherein said baffle system includes an insert having a central stem with one or more ribs extending out radially from the stem.

13. The pipette tip device as recited in claim **12**, wherein said stem is formed by two intersecting ribs.

14. The pipette tip device as recited in claim **12**, wherein said stem is formed by more than two intersecting ribs.

15. The pipette tip device as recited in claim **1**, wherein said baffle system includes at least one ridge along the interior surface of said pipette tip.

16. The pipette tip device as recited in claim **1**, wherein said baffle system includes four, evenly spaced ridges along the interior surface of said pipette tip.

17. The pipette tip device as recited in claim **15**, wherein said at least one ridge is linear.

18. The pipette tip device as recited in claim **15**, wherein said at least one ridge is spiral.

19. The pipette tip device as recited in claim **15**, wherein said at least one ridge is annular.

20. The pipette tip device as recited in claim **1**, wherein said baffle system is about parallel to the cylindrical axis of said pipette tip.

21. A pipette tip device for performing dispersive SPE, comprising:

a pipette tip having a lower barrier and an upper barrier;

sorbent material contained between said lower barrier and said upper barrier; and

a baffle system contained within said pipette tip and above said sorbent material, wherein said baffle system is shaped to disrupt liquid flow when a liquid sample is aspirated into said pipette tip.

22. The pipette tip device as recited in claim **21**, wherein said baffle system includes a surface that is able to perform an immunoassay screen.

23. The pipette tip device as recited in claim **21**, wherein said baffle system includes a surface that is able to measure pH.

24. The pipette tip device as recited in claim **21**, wherein said baffle system includes a surface that is able to detect adulterants.

25. A method for performing dispersive SPE, comprising the steps of:

providing a pipette tip device, having a lower barrier; sorbent material contained above said lower barrier; and a baffle system contained within said pipette tip;

providing a sample solution;

aspirating said sample solution into said pipette tip device; and

mixing said sorbent material with said sample solution to perform an SPE extraction, wherein said baffle system is shaped to disrupt said sorbent material when in solution.

26. The method as recited in claim **25**, wherein said pipette tip is had a volume of about 1 mL or less.

27. A method for performing dispersive SPE, comprising the steps of:

providing a pipette tip device, having a lower barrier; sorbent material contained above said lower barrier; and a baffle system contained within said pipette tip;

providing a sample solution;

aspirating said sample solution into said pipette tip device;

mixing said sorbent material with said sample solutions to perform an SPE extraction, wherein said baffle system is shaped to disrupt said sorbent material when in solution;

dispensing the extracts from said SPE extraction to waste;

aspirating wash solvent into said pipette tip device;

mixing said sorbent material with said wash solvent;

dispensing said wash solvent to waste;

aspirating elution solvent into said pipette tip device;

mixing said sorbent with elution solvent; and

collecting the resulting eluate from said SPE extraction.

* * * * *