

VARIAN, INC.

Sample Preparation Products

Certify Methods Manual

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EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY



SUMMARY OF CERTIFY/CERTIFY II MIXED MODE EXTRACTION

section 1 - GENERAL INFORMATION

In screening for drugs of abuse in biological fluids high; purity, high recovery, and rugged methods are essential for an effective screen and to avoid false positives. The Certify mixed mode sorbent takes advantage of non-polar, polar, and ion exchange properties to ensure rapid, reproducible, simple, and clean extraction of many drug classes. Because the Certify sorbent is capable of exhibiting a variety of sorbent-analyte interactions, it can be used either as a general screen for several broad drug classes or in specific extractions for GC and GC/MS confirmation of drugs and metabolites.

The multi-faceted performance of Certify arises from its mixed mode composition. The use of a bonded phase containing a medium-length hydrocarbon chain allows for some exposure of the polar silica surface. Therefore polar and non-polar interactions of the drugs and matrix interferences with the sorbent are optimized. The second bonded phase, a strong cation exchanger, has also been optimized for capacity. Too many ion exchange sites would result in high background and difficult elution, whereas too few would produce low recoveries. The complex sorbent is specially tested, first through rigorous ion exchange and non-polar chromatographic checks of the component phases, then through a final drug screen using the completed product. Because the three modes of retention – polar, non-polar, ion exchange – are precisely matched, Certify is ideal for general drug screening or the extraction of specific basic, acidic, or neutral drugs. What follows is a general discussion of how each drug class interacts with the Certify sorbent to allow for its extraction from complex biological matrices.

section 2 - INITIAL CONSIDERATIONS

The Certify cartridge is conditioned first with methanol to open up the coiled hydrophobic portion of the sorbent and activate it toward interaction with a polar matrix. Further conditioning with buffer removes excess methanol and places the sorbent bed in an environment as similar to the matrix as possible. This allows for maximum sorbent-matrix interaction and reproducible recoveries. The extraction of drugs from a complex biological matrix such as plasma or urine requires that pH, ionic strength, and viscosity be controlled. This is accomplished by dilution of the sample with buffer.

section 3 - BASIC DRUGS

(amphetamines, phencyclidine, proxyphene, meperidine, LSD, codeine, oxycodone, opiates)

Although very different in their pharmacology and structure, all basic drugs feature an amine functional group (NR_3 , NR_2H , or NRH_2). This group acts as a base by abstracting H^+ and becoming positively charged. Initial extraction, however, takes place by a non-polar mechanism onto the hydrophobic portion of the sorbent. After the drug is retained, washing the cartridge with water removes polar interferences. Next, the cartridge is washed with acid, completing the elution of polar interferences and ensuring that the basic drugs are positively charged as ammonium salts. Non-polar, non-basic drugs and interferences can then be removed with an organic wash. The presence of water during the organic wash negatively affects the efficiency of the wash by minimizing contact of the sorbent with the organic solvent. Therefore, it is important that the sorbent is thoroughly dried to remove any residual water before the organic wash. Finally, the basic drugs can be eluted with an alkaline organic solvent (i.e. 2% NH_4OH in either methanol, EtOAc, or $\text{CH}_2\text{Cl}_2/\text{IPA}$). The presence of base serves to disrupt the ionic interactions of the drug with the sorbent as the positive charge on the drug is neutralized. The use of organic solvent disrupts the hydrophobic interactions which initially retained the drug from the sample.

section 4 - ACIDIC AND NEUTRAL DRUGS

(barbiturates, phenytoin, methaqualone, benzodiazapines, Δ^9 -carboxy THC)

As with basic drugs, acidic and neutral drugs have widely varying pharmacological and structural properties. They are classed together because they are not retained by a cation exchange mechanism, although the cation exchange portion of Certify can improve clean-up of samples containing these drugs. These drugs are characterized by the absence of a basic amine functional group. (Note, drugs such as barbiturates contain a nitrogen-containing *imine* functional group, which is weakly acidic, rather than basic.) These drugs are retained by a non-polar mechanism. Washing the cartridge with dilute acid removes polar impurities and ensures that any basic interferences become charged. Thus, when the acidic and neutral drugs are eluted by disrupting their non-polar interaction with the sorbent, the basic interferences are retained on the strong cation-exchange portion of the sorbent.

Certify II is a mixed mode sorbent originally developed for the cannabinol derivative Δ^9 -carboxy THC. Because this drug contains an acidic functional group, clean-up from urine samples can be optimized by using an *anion* exchange sorbent, rather than the cation exchanger found in Certify. As with basic drugs on Certify, retention of acidic drugs on Certify II is initially achieved by non-polar interactions on the hydrophobic portion of the sorbent. Next, polar interferences can be washed away with a basic buffer. This wash step also ensures that the COOH functional group is deprotonated, forming COO^- , which can then be retained on the anion exchange portion of the Certify II sorbent. The charge on any amine functional groups would be neutralized by this step as well, preparing any basic drugs present for washing. After briefly drying the cartridge, non-polar basic drugs and interferences can be removed with a non-polar solvent. Finally, the Δ^9 -carboxy THC can be recovered by elution with a non-polar acidic solvent such as hexane/ethyl acetate with 1% acetic acid.

Many of the methods contained in this booklet have been successfully employed by the largest workplace testing laboratories throughout the world for nearly 20 years.

Many laboratories have made modifications of these methods to suit their specific needs or requirements. In addition, with the advent of more sensitive detectors, the desire to analyze additional drug metabolites, the need for increased throughput and advancements in chromatography techniques, further modifications have been used by many facilities.

The following information should aid you in making modifications to these methods to suit specific analytical requirements. Should you have additional questions about these methods or modification considerations please contact our Technical HelpDesk at 1-800-926-3000 or helpdesk.us@varianinc.com.

Cartridge bed mass

These methods and modifications are designed for Bond Elut Certify and Certify II cartridge methods suggested in the manual. Standard Bond Elut Certify methods (strong cation exchange and C8) uses a 130 mg bed mass. Bond Elut Certify II (strong anion exchange and C8) uses a 200 mg bed mass. Smaller sample sizes can lead to the use of smaller bed masses and in turn, decreasing rinse and elution volumes, and potentially, increasing throughput. Varian's technical support team would be pleased to discuss these options with you.

Sample Size

With significant improvements in chromatography and detector technology, a 5 mL urine sample is typically no longer necessary. 1-3 mL is often sufficient for typical levels of detection. The volume of accompanying buffer can be proportionally altered, or for ease of use, remain the same at 2 mL buffer. 2 mL of urine matrix with 2 mL of phosphate buffer are typical sample volumes.

Conditioning

2 mL MeOH followed by 2 mL of buffer are still considered standard for conditioning. Labs sometimes use gravity flow at this step to ensure that the SPE beds do not go to dryness. To facilitate faster methods or reduce solvent usage, 500 μ L of MeOH can be used. Apply the MeOH with approximately 2" Hg vacuum until the MeOH has entered the columns completely. Gravity may also be used. Follow the MeOH with 1 mL of specified buffer and allow the buffer to pass through the cartridges. Because the buffer is also used to dilute the urine samples, residual buffer above the top frit of the SPE

cartridge is acceptable. *Avoid high levels of vacuum and extensive drying times prior to the addition of the sample. This can cause de-conditioning of the cartridge and can affect recoveries.* When working with serum/plasma samples, 1-2 mL buffer is a typical volume for removing residual MeOH in the conditioning step. This prevents protein precipitation as the sample passes through the cartridge.

Column Rinsing and Drying

Methods may be modified such that no individual rinse step is more than 3 mL. Note that drying the cartridges is often recommended between column rinses or prior to elution. In some cases, such as THC and its metabolite, over-drying can lead to diminished recoveries. For THC, 10-15” Hg of vacuum for 2-3 minutes is recommended where noted in the method. For most methods, drying prior to elution facilitates the rapid elution of compounds from the cartridge and is recommended.

Elution

Unless specified, 2 mL is an optimal volume. To optimize recovery, elution should be performed in multiple aliquots (2 x 1 mL). This does increase the number of steps required in the method.

Derivatization

The options for the types of derivatives are too numerous to mention here. BSTFA is a very rugged and often-used derivatization reagent. Other derivatives are employed to move peaks of interest away from interfering peaks, thus maintaining proper ion ratios. Derivatives are also used to increase compound mass and sensitivity with MS detectors.

Alternative matrices

Hair, oral fluids and tissue matrices are routinely tested in many laboratories. Visit our website www.varianinc.com, for all new and updated sample prep and instrumentation methods for these newer matrices.

Other resources:

The Varian Toxicology Manual offers some additional approaches for sample prep. Contact the technical helpdesk or your local sales representative for additional information.

BOND ELUT CERTIFY/CERTIFY II
PART NUMBERS



Certify and Certify II are available in a wide variety of cartridge volumes to accommodate both manual and automated drug extraction methods. Sorbent masses from 50 mg to 1 g are available for both standard drug testing methods and pharmaceutical research.

Certify and Certify II are also available in a 96-well format. Contact Varian's Technical HelpDesk at 800-926-3000, for more information and a current list of available configurations, part numbers, and prices.

**EXTRACTION OF DRUGS OF ABUSE
USING BOND ELUT CERTIFY**



***SOLVENTS, SOLVENT MIXTURES, REAGENTS, AND SOLUTIONS
USED IN CERTIFY AND CERTIFY II EXTRACTIONS***

section 1 - SOLVENTS

Acetone: HPLC Grade

Acetonitrile (CH₃CN): HPLC Grade

Chloroform (CHCl₃): HPLC Grade

Distilled or Deionized Water (DI H₂O): 5 ≤ pH ≤ 7

Ethyl Acetate (EtOAc): HPLC Grade

Hexane: HPLC Grade

Isopropyl Alcohol (IPA): HPLC Grade

Methanol (CH₃OH or MeOH): HPLC Grade

Methylene Chloride (CH₂Cl₂ or MeCl₂): HPLC Grade

section 2 - SOLVENT MIXTURES

Acetone/Chloroform (50/50)

Acetonitrile/DI H₂O (25/75)

CH₂Cl₂/IPA (80/20)

Hexane/Ethyl Acetate (50/50)

Hexane/Ethyl Acetate (80/20)

Methanol/DI H₂O (10/90)

NOTES:

Storage of organic solvents in some plastic containers may lead to contamination of the solvent or solvent mixture by plasticizers, which may interfere with analyte quantitation.

Good laboratory practice dictates that those who handle or are potentially exposed to reagents, solvents, and solutions used or stored in the laboratory should familiarize themselves with manufacturer's recommendations for chemical storage, use, and handling, and should also familiarize themselves with an appropriate Material Safety Data Sheet (MSDS) for each material for which a Material Safety Data Sheet exists.

section 3 - REAGENTS

Acetic Acid, Glacial (CH_3COOH): 17.4 M

Ammonium Hydroxide (NH_4OH): concentrated (14.8 M)

β -Glucuronidase: lyophilized powder from limpets (*Patella Vulgatta*)

Dimethylformamide (DMF): silylation grade

Hydrochloric Acid (HCl): concentrated (12.1 M)

N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) with 3% trimethylsilyliodide

N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS)

Pentafluoropropionic Acid Anhydride (PFPA)

Phosphoric Acid (H_3PO_4): concentrated (14.7 M)

Potassium Bicarbonate (KHCO_3): F.W. 100.12

Potassium Hydroxide (KOH): F.W. 56.11

Potassium Phosphate Monobasic (KH_2PO_4): F.W. 136.09

Sodium Acetate (CH_3COONa): F.W. 82.03

Sodium Acetate Trihydrate ($\text{CH}_3\text{COONa}\cdot 3\text{H}_2\text{O}$): F.W. 136.08

TRIS Base

section 4 - SOLUTIONS

Acetic Acid, 1.0 M:

To 400 mL DI H₂O add 28.6 mL glacial acetic acid. Dilute to 500 mL with DI H₂O. Mix.
storage: 25 °C in glass or plastic
stability: 6 months

Acetic Acid, 0.01 M:

Dilute 57.5 µL glacial acetic acid to 100 mL with DI H₂O. Mix.
storage: 25 °C in glass or plastic
stability: 6 months

Acetic Acid, 100 mM:

Dilute 40 mL 1.0 M acetic acid to 400 mL with DI H₂O. Mix.
storage: 25 °C in glass or plastic
stability: 6 months

Acetate Buffer, 100 mM (pH 4.0):

Into a 100 mL volumetric flask add 80 mL DI H₂O. To this, add 570 µL of glacial acetic acid. Mix. Add 1.6 mL of 1.0 M KOH. Check pH. The pH should be 4.0. Adjust the pH to 4.0 if necessary. Make up to volume with DI H₂O. Mix well.
storage: 25 °C in glass or plastic
stability: 6 months; Inspect daily with use for contamination.

Acetate Buffer, 1.0 M (pH 5.0):

Dissolve 42.9 g sodium acetate trihydrate in 400 mL DI H₂O; add 10.4 mL glacial acetic acid. Dilute to 500 mL with DI H₂O. Mix. Adjust pH to 5.0 ± 0.1 with 1.0 M sodium acetate or 1.0 M acetic acid.
storage: 25 °C in glass or plastic
stability: 6 months; Inspect daily with use for contamination.

β-Glucuronidase, 5,000 Fishman units/mL:

Dissolve 100,000 Fishman units lyophilized powder with 20 mL acetate buffer, 1.0 M (pH 5.0).
storage: -5 °C in plastic
stability: Several days; prepare daily for best results.

Ethyl Acetate/Ammonium Hydroxide (98/2):

To 98 mL EtOAc add 2 mL concentrated NH₄OH. Mix
storage: 25 °C in glass
stability: 1 day

Hydrochloric Acid, 100 mM:

To 400 mL DI H₂O add 4.2 mL concentrated HCl. Dilute to 500 mL with DI H₂O. Mix.
storage: 25 °C in glass or plastic
stability: 6 months

Hydrochloric Acid, 1.0 M:

Into a 100 mL volumetric flask add 50 mL DI H₂O. To this, add 8.3 mL of concentrated HCl. Bring to volume with DI H₂O.
storage: 25 °C in glass
stability: 1 day

Methanol/Ammonium Hydroxide (98/2):

To 98 mL MeOH add 2 mL concentrated NH₄OH. Mix
storage: 25 °C in glass or fluoropolymer plastic
stability: 1 day

Methylene Chloride/Isopropanol/Ammonium Hydroxide (78/20/2):

To 20 mL IPA add 2 mL concentrated NH₄OH. Mix. Add 78 mL CH₂Cl₂. Mix.
storage: 25 °C in glass or fluoropolymer plastic
stability: 1 day

Phosphate Buffer, 100 mM (pH 6.0):

Weigh 13.6 g of KH₂PO₄ into a 1.0 L volumetric flask. Dissolve the KH₂PO₄ into 900 mL DI H₂O. Adjust pH to 6.0 (± 0.1) with 1.0 M KOH while stirring. Bring to total volume up to 1.0 L with DI H₂O.
storage: 5 °C in glass
stability: 1 month; Inspect daily with use for contamination.

Phosphoric Acid 50 mM:

Add 3.4 mL of phosphoric acid to 950 mL DI H₂O in a 1.0 L volumetric flask. Mix and bring to volume with DI H₂O.
storage: 25 °C in glass or plastic
stability: 6 months

Potassium Hydroxide, 1.0 M:

Weigh 5.6 g KOH into a clean plastic 100 mL volumetric flask. Dissolve the KOH with DI H₂O and bring to volume.
storage: 25 °C in plastic
stability: 6 months

Potassium Hydroxide, 10.0 M:

Into a 250 mL plastic volumetric flask add 150 mL DI H₂O. To this, add 140 g KOH. Dissolve the KOH and bring to 250 mL with DI H₂O.

storage: 25 °C in plastic

stability: 3 months

Sodium Acetate, 1.0 M:

Dissolve 13.6 g sodium acetate in 90 mL DI H₂O. Dilute to 100 mL with DI H₂O. Mix.

storage: 25 °C in glass or plastic

stability: 6 months

TRIS Buffer, 2.0 M (pH 8.1):

Weigh 242.2 g TRIS base (tris[hydroxymethyl]aminomethane) in a 1.0 L volumetric flask. Dissolve TRIS base in 900 mL DI H₂O. Adjust the pH to 8.1 with 1.0 M HCl while stirring. Bring the total volume to 1.0 L with DI H₂O. (NOTE: TRIS base is also known as TRIZMA base or THAM. TRIZMA HYDROCHLORIDE (tris[hydroxymethyl]aminomethane hydrochloride) can also be used to prepare the buffer. A 2.0 M solution requires 315.2 g of TRIZMA HCl, and NaOH is required to bring the acidic solution to a pH of 8.1.

storage: 25 °C in glass or plastic

stability: 30 days

***EXTRACTION OF DRUGS OF ABUSE
USING BOND ELUT CERTIFY***

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ANALYTE NAME/MATRIX M2707 Amphetamines in Urine	ANALYTICAL TECHNIQUE GC or GC/MS	PRODUCT/PART NUMBER USED 130 mg CERTIFY 1210-2051 or 1211-3050	
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section 1 - PRINCIPLE AND MECHANISMS

Basic drug extraction utilizing both non-polar and cation exchange mechanisms.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

To 5 mL of urine add internal standard(s)* and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

- a) 2 mL CH₃OH; draw through under vacuum.
 - b) 2 mL 0.1 M phosphate buffer (pH 6.0); draw through under vacuum.
- NOTE:** Use a low vacuum (≤ 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

3. COLUMN RINSE

- a) 1 mL 1.0 M acetic acid; draw through under vacuum.
- b) Dry column for 5 minutes under vacuum.
- c) 6 mL CH₃OH; draw through under vacuum.
- d) Dry column (2 minutes at ≥ 10 inches Hg).

5. ELUTE AMPHETAMINES

2 mL CH₂Cl₂/IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

6. CONCENTRATE ELUATE

- a) Add 100 μ L silylation grade DMF to eluate.
- b) Evaporate to 100 μ L at $\leq 40^\circ\text{C}$.

* Suggested internal standards for GC/MS: *d*₅-Amphetamine and *d*₅-Methamphetamine.
Suggested internal standards for GC/FID: phentermine, propylamphetamine, other amphetamine analogs

DERIVATIZATION:

1. Add 50 μ L HFBA (heptafluorobutyric anhydride).
2. React 20 minutes at room temperature

section 3 - ANALYSIS

Inject 1 to 3 μ L into chromatograph. Monitor the following ions:

<u>Amphetamine</u>	<u>d₅-Amphetamine</u>	<u>Methamphetamine</u>	<u>d₅-Methamphetamine</u>
240**	245**	254**	259**
91	91	210	210
118	123	118	123

** Quantitation ion

section 4 - OTHER EXTRACTION INFORMATION

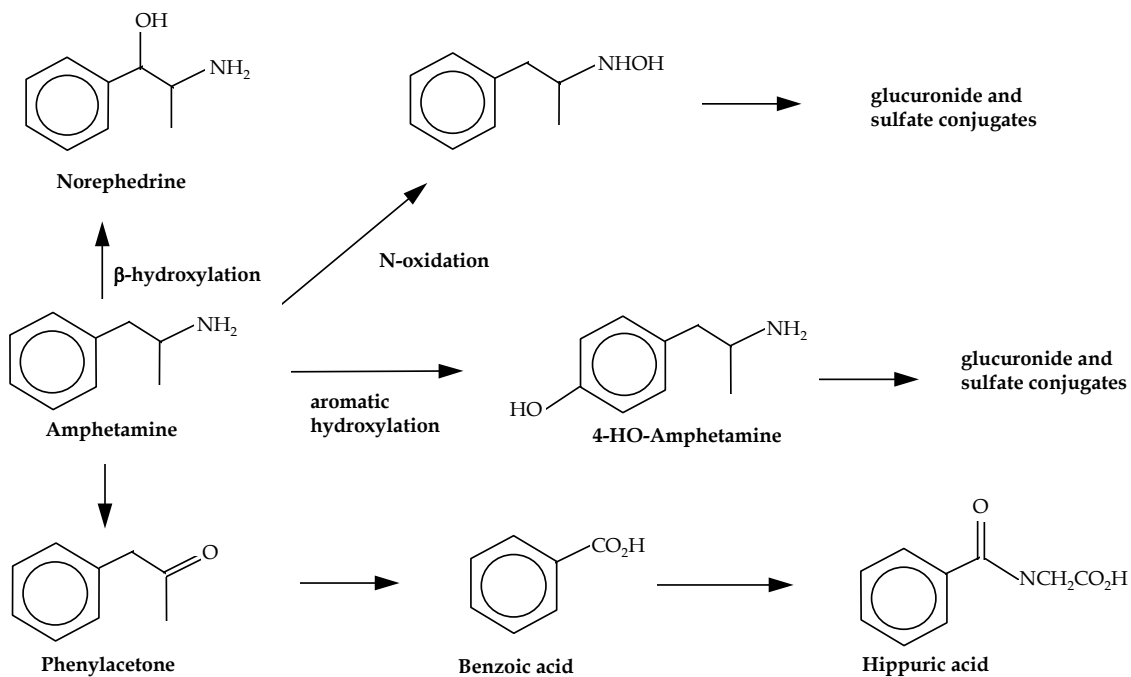
Free bases of amphetamines are volatile. An alternative to the above evaporation procedure above is to add 50 μ L methanolic HCl (MeOH:conc. HCl; 9:1; v/v) to the eluent before evaporation. This forms the corresponding (nonvolatile) hydrochloride salts of the drugs.

section 5 - METABOLISM AND EXCRETION

- Plasma elimination half life is 8-12 h.
- Drugs appear in urine within 20 minutes of administration.
- Amphetamine is excreted as unchanged drug and as deaminated and hydroxylated metabolites (hippuric, benzoic acids).
- Rate and proportions of excreted compounds vary considerably with pH of urine.
- Recommended target analytes are unchanged drugs.

For method optimization tips, see p. 8

METABOLIC PATHWAY



section 6 - OTHER INFORMATION

Positive amphetamine analysis generally indicates use within previous 24-48 h. Several non-proprietary drug preparations used as decongestants and anorectics contain ephedrine and phenylpropanolamine, which can produce positive immunoassay tests. Some prescription drugs (benzphetamine, fenfluramine, mephentermine, phenmetrazine, phentermine) can also produce positive immunoassay results. Some drugs give amphetamine and methamphetamine in urine as metabolites.

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY

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Analyte Name/Matrix

M2708

Anabolic Steroids in Urine

ANALYTICAL TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER USED

130 mg CERTIFY

1210-2051 or 1211-3050

section 1 - PRINCIPLE AND MECHANISMS

Drug extraction using hydrophobic interactions for retention and ion exchange and secondary polar interactions to remove interferences.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

β-GLUCURONIDASE HYDROLYSIS:

To 5 mL of urine add internal standard(s)* and 2 mL of β-glucuronidase (5,000 F units/mL Patella Vulgata in 1.0 M acetate buffer, pH 5.0). Mix/vortex. Hydrolyze for 3 hours at 65°C. Cool before proceeding. Adjust sample pH to 6.0 ± 0.5 with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. PREPARE CERTIFY EXTRACTION COLUMN

- a) 3 mL CH₃OH; draw through under vacuum.
- b) 3 mL DI H₂O; draw through under vacuum.
- c) 1 mL 100 mM phosphate buffer (pH 6.0); draw through under vacuum.

NOTE: Use a low vacuum (≤ 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

3. COLUMN RINSE

- a) 3 mL 10% (v/v) CH₃OH in DI H₂O; draw through under vacuum.
- b) Dry column (5 minutes at ≥ 10 inches Hg).
- c) 1 mL hexane or hexane/ethyl acetate (50/50); draw through under vacuum.

4. ELUTE ANABOLIC STEROIDS (Choose a, b, c or d)
- a) 3 mL CH₂Cl₂/IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute.
NOTE: Prepare elution solvent daily.
 - b) 3 mL CH₂Cl₂/IPA (80/20)
 - c) 3 mL ethyl acetate
 - d) 3 mL CH₃OH

5. DRY ELUATE

Evaporate to dryness at ≤ 40°C.

DERIVATIZATION:

Add 50 µL MSTFA (with 3% trimethylsilyliodide). Mix/vortex. React 20 minutes at 70°C. Remove from heat source to cool.

NOTE: Do not evaporate MSTFA solution.

section 3 - ANALYSIS

Inject 1 to 3 µL sample (in MSTFA solution) into GC.

Principle Ions (Mass Selective Detection):

Testosterone-TMS: 432,301,209	11-B-Hydroxyandosterone: 522,417,158
19-Noretiocholanone-TMS: 405,315,225	Methandienone: 409,313,281
Oxymetholone: 640,552,462,370,143	19-Norandosterone-2TMS: 405,315,225
Dehydroepiandrosterone-2TMS: 432,327,297,169	16-A-Hydroxyetiocholanone-TMS: 504,417
10-Nortestosterone-2TMS: 418,287,194	17-A-Epitestosterone-TMS: 432,341,327,209
Oxymetholone metab. #1: 640,552,462,143	Stanozolol-TMS: 472,381,342,149
Oxymetholone metab. #2: 625,462,370,143	

For method optimization tips, see p. 8

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY



ANALYTE NAME/MATRIX	ANALYTICAL TECHNIQUE	PRODUCT/PART NUMBER USED
M2709 Barbiturates in Urine	GC or GC/MS	130 mg Certify 1210-2051 or 1211-3050

section 1 - PRINCIPLE AND MECHANISMS

Drug extraction using hydrophobic interactions for retention and ion exchange and secondary polar interactions to remove interferences.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

To 5 mL of urine add internal standard(s)* and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum.

2 mL 100 mM phosphate buffer (pH 6.0); draw through under vacuum.

NOTE: Use a low vacuum (≤ 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

3. COLUMN RINSE

a) 1 mL 100 mM phosphate buffer (pH 6.0)/CH₃OH (80/20); draw through under vacuum.

b) Dry for 5 minutes under vacuum (≥ 10 inches Hg).

c) 1 mL 1.0 M acetic acid; draw through under vacuum.

d) Dry cartridge for 10 minutes under vacuum.

e) 1 mL hexane; draw through under vacuum.

f) Dry cartridge for 2 minutes under vacuum.

4. ELUTE BARBITURATES

4 mL hexane/ethyl acetate (75/25); collect eluate at ≤ 5 mL/minute.

5. DRY ELUATE

Evaporate to dryness at $\leq 40^{\circ}\text{C}$. Reconstitute with 100 μL ethyl acetate.

***section 3* - ANALYSIS**

Inject 1 to 2 μL into chromatograph.

Monitor the following ions (Mass Selective Detection):

<u>Amobarbital</u>	<u>Butobarbital</u>	<u>Butalbital</u>	<u>Hexobarbital</u>
156**	156**	168**	221**
141	141	153	157
157	157	141	156
<u>Pentobarbital</u>	<u>Phenobarbital</u>	<u>Secobarbital</u>	
156**	204**	168**	
141	117	153	
157	232	195	

* Suggested internal standard for GC/MS: Hexobarbital

** Quantitation ion

For method optimization tips, see p. 8

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY

VARIAN 

ANALYTE NAME/MATRIX

M2710

Basic Drugs from Urine

ANALYTICAL
TECHNIQUE

HPLC

PRODUCT/PART NUMBER USED

130 mg CERTIFY

1210-2051 or 1211-3050

section 1 - PRINCIPLE AND MECHANISMS

Basic drug extraction utilizing both non-polar and cation exchange mechanisms.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

To 5 mL of urine add internal standard(s) and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum.

2 mL DI H₂O; draw through under vacuum.

1 mL 100 mM phosphate buffer (pH 6.0); draw through under vacuum.

NOTE: Use a low vacuum (≤ 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

3. COLUMN RINSE

2 mL DI H₂O; draw through under vacuum.

2 mL 100 mM HCl; draw through under vacuum.

3 mL CH₃OH; draw through under vacuum.

Dry column (5 minutes at ≥ 10 inches Hg).

4. ELUTE BASES

2 mL CH₃OH/NH₄OH (98/2); collect eluate at 1 to 2 mL/minute.

5. EVAPORATE

Evaporate to dryness at $\leq 40^\circ\text{C}$.

section 3 - ANALYSIS

Reconstitute in mobile phase and inject into chromatograph.

For method optimization tips, see p. 8

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY

VARIAN



ANALYTE NAME/MATRIX

M2711

Benzodiazepines in Serum Or Plasma

ANALYTICAL
TECHNIQUE

HPLC

PRODUCT/PART NUMBER USED

130 mg CERTIFY

1210-2051 or 1211-3050

section 1 - PRINCIPLE AND MECHANISMS

Drug extraction using hydrophobic interactions for retention and ion exchange and secondary polar interactions to remove interferences.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

To 1 mL serum or plasma add internal standard and 1.0 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum.

2 mL DI H₂O; draw through under vacuum.

1 mL 100 mM phosphate buffer (pH 6.0); draw through under vacuum.

NOTE: Use a low vacuum (≤ 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

3. COLUMN RINSE

3 mL DI H₂O; draw through under vacuum.

1 mL 100 mM HCl or 1.0 M acetic acid; draw through under vacuum.

3 mL CH₃OH; draw through under vacuum.

Dry column (5 minutes at ≥ 10 inches Hg).

4. ELUTE BENZODIAZEPINES

2 mL CH₂Cl₂/IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

5. DRY ELUATE

Evaporate to dryness at $\leq 40^\circ\text{C}$.

section 3 - ANALYSIS

Reconstitute in mobile phase.

Inject sample into chromatograph.

For method optimization tips, see p. 8

EXTRACTION OF DRUGS OF ABUSE FROM URINE USING BOND ELUT CERTIFY

VARIAN



ANALYTE NAME/MATRIX

M2712

Benzodiazepines in Urine

ANALYTICAL
TECHNIQUE

GC or GC/MS

PRODUCT/PART NUMBER USED

130 mg CERTIFY

1210-2051 or 1211-3050

section 1 - PRINCIPLE AND MECHANISMS

Drug extraction using hydrophobic interactions for retention and ion exchange and secondary polar interactions to remove interferences.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

β-GLUCURONIDASE HYDROLYSIS

To 5 mL of urine add internal standard(s)* and 2 mL of β-glucuronidase (5,000 F units/mL *Patella Vulgata* in 1.0 M acetate buffer, pH 5.0). Mix/vortex. Hydrolyze for 3 hours at 65°C. Cool before proceeding.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum.

2 mL DI H₂O; draw through under vacuum.

1 mL 100 mM phosphate buffer (pH 6.0); draw through under vacuum.

NOTE: Use a low vacuum (≤ 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

3. COLUMN RINSE

2 mL DI H₂O; draw through under vacuum.

2 mL 20% acetonitrile in 100 mM phosphate buffer (pH 6.0); draw through under vacuum.

Dry column (5 minutes at ≥ 10 inches Hg).

2 mL hexane; draw through under vacuum.

4. ELUTE BENZODIAZEPINES

2 mL CH₂Cl₂/IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute.

5. DRY ELUATE

Evaporate to dryness at ≤ 40°C.

DERIVATIZATION:

Add 50 µL BSTFA (with 1% TMCS) and cap. Mix/vortex. React 20 minutes at 70 °C. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA solution.

section 3 - ANALYSIS

Inject 1 to 3 µL sample (in BSTFA solution) into chromatograph.

Principle ions (Mass Selective Detection):

Alprazolam 308**,279,204	Temazepam (TMS) 343**,283,257
Clonazepam 387**,352,306	Chlordiazepoxide 282**,283,284
Desalkylflurazepam (TMS) 359**,341,245	α-Hydroxytriazola 415*,17,430
Diazepam 256**,283,221	α-Hydroxyalprazolam 381**,396,383
Halazepam 324**,352,289	Hydroxyethylflurazepam 288**,287, 289
Lorazepam (TMS) 429**,430,347	Triazolam 313**,314,342
Nordiazepam (TMS) 341**,342,343	Prazepam 269**,241,324
Oxazepam (TMS) 429**,430,313	4-Hydroxydiazepam 86**,109,307

* Suggested internal standard for GC/MS: Prazepam, *d*₅-Oxazepam

** Quantitation ion

section 4 - OTHER INFORMATION

Note: Flurazepam does not extract under these conditions; however, the metabolites such as desalkylflurazepam and hydroxyethyl-flurazepam will extract with high recovery. A basic wash is necessary in order to recover flurazepam; however, this reduces the recovery of other benzodiazepines.

For method optimization tips, see p. 8

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY

VARIAN 

ANALYTE NAME/MATRIX	ANALYTICAL TECHNIQUE	PRODUCT/PART NUMBER USED
M2713 Cocaine And Benzoyllecgonine in Serum, Plasma, Or Whole Blood	GC or GC/MS	130 mg Certify 1210-2051 or 1211-3050

section 1 - PRINCIPLE AND MECHANISMS

Basic drug extraction utilizing both non-polar and cation exchange mechanisms. At neutral pH, benzoyllecgonine carries both a positive and a negative charge, therefore acidification of the sample is necessary to neutralize the acidic functional group for reproducible cation exchange at the amine functional group.

section 2 - EXTRACTION METHOD

SAMPLING PROCEDURE:

Target analytes show poor hydrolytic stability, particularly under alkaline conditions. Samples should be kept cool and dark as much as possible after collection. Blood samples are best preserved with fluoride and kept at a pH of 5.

SAMPLE PREPARATION:

A. Serum or Plasma:

To 1 mL of serum or plasma add internal standard(s)* and 4 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

B: Whole Blood:

See Appendix A for specimen treatment. Dilute 1 part resulting supernatant with 4 parts 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum.

2 mL 100 mM phosphate buffer (pH 6.0); draw through under vacuum.

NOTE: Use a low vacuum (≤ 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute

- Suggested internal standards for GC/MS: *d*₃-Cocaine, *d*₃-Benzoyllecgonine. Suggested internal standards for GC/FID: a) analogues of benzoyllecgonine (propylbenzoyllecgonine), b) opiate alkaloids (levallorphan, nalorphine, ethylmorphine, codeine), c) misc. (*n*-tetracosane, tetraphenylethylene (FID only), butylanthraquinone)

3. COLUMN RINSE

- 6 mL DI H₂O; draw through under vacuum.
- 3 mL 1 M acetic acid; draw through under vacuum.
- Dry for 5 minutes under vacuum.
- 6 mL CH₃OH; draw through under vacuum.

4. ELUTE COCAINE AND BENZOYLECGONINE

- 2 mL CH₂Cl₂/IPA (80:20) containing 2% NH₄OH; collect eluate at 1 to 2 mL/minute.
- NOTE:** Prepare elution solvent daily.

5. DRY ELUATE

- Evaporate to dryness at ≤ 40°C.

DERIVATIZATION:

Add 50 µL BSTFA (with 1% TMCS) and cap. Mix/vortex. React 20 minutes at 70°C. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA solution.

PFPA derivatization is also acceptable.

section 3 - ANALYSIS

Inject 1 to 3 µL sample (in BSTFA solution) into chromatograph.
Monitor the following ions (Mass Selective Detection):

<u>Cocaine</u>	<u>d₃-Cocaine</u>	<u>TMS-BE</u>	<u>TMS- d₃-BE</u>
182**	185**	240**	243**
198	201	256	259
303	306	361	364

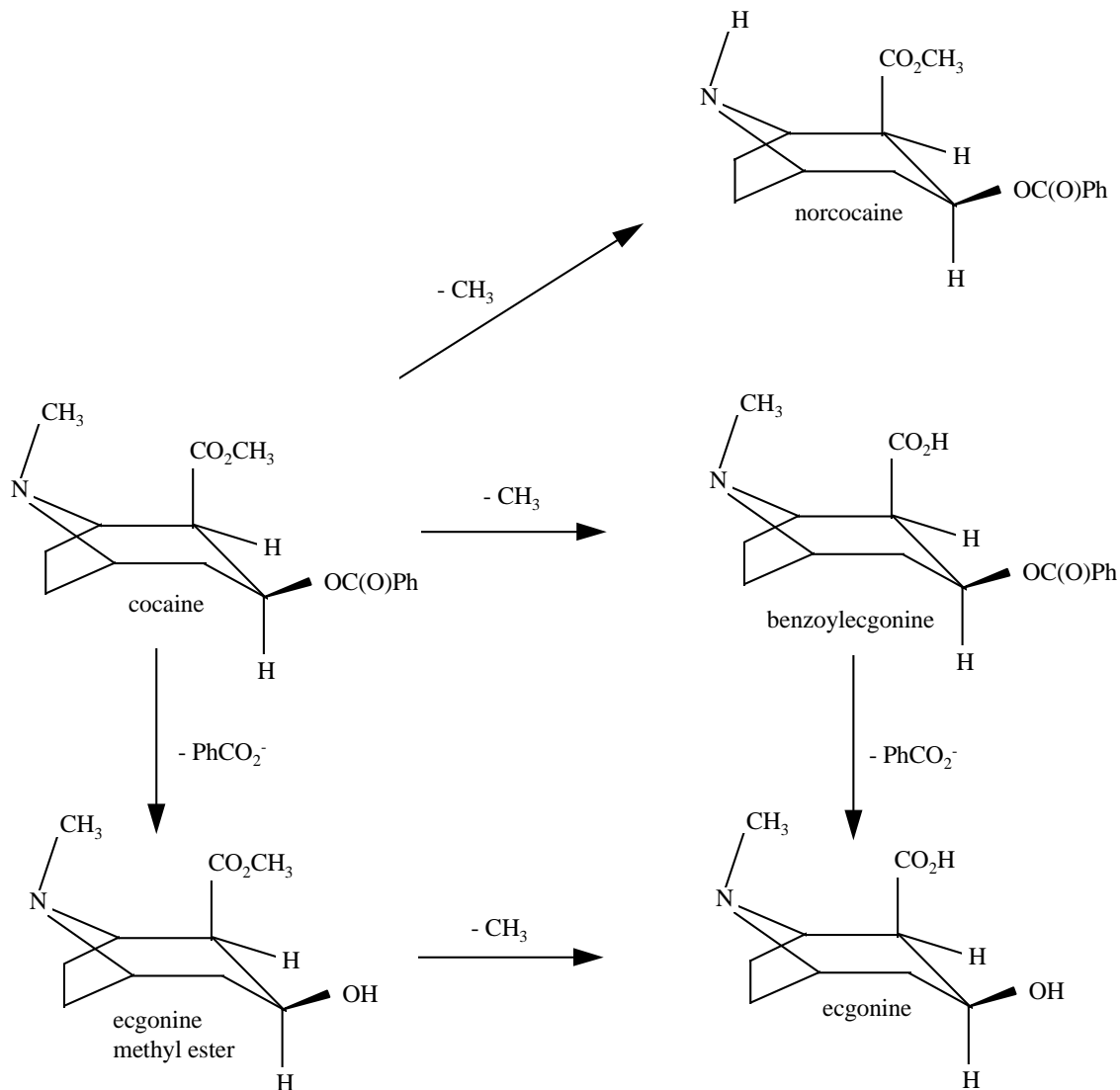
** Quantitation ion

For method optimization tips, see p. 8

section 4 - METABOLISM AND EXCRETION

- Eliminated in urine as unchanged drug (1-9% of dose), benzoylecgonine (35-54%), and ecgonine methyl ester (32-49%).
- Norcocaine is a minor metabolite.
- Pattern of metabolite excretion can be altered by abnormalities of pseudocholinesterase activity (enzyme which forms ecgonine methyl ester).
- After a single dose of cocaine the unchanged drug can be detected for up to 24 h.
- After chronic use, detection time is up to 5 days or more.
- Minimal differences are found in relative amounts of metabolites excreted following administration of cocaine intranasally, intravenously, or by smoking.

METABOLIC PATHWAY



EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY

VARIAN 

ANALYTE NAME/MATRIX	ANALYTICAL TECHNIQUE	PRODUCT/PART NUMBER USED
M2714 Cocaine And Benzoyllecgonine In Serum, Plasma, Or Whole Blood	HPLC	130 mg Certify 1210-2051 or 1211-3050

section 1 - PRINCIPLE AND MECHANISMS

Basic drug extraction utilizing both non-polar and cation exchange mechanisms. At neutral pH, benzoyllecgonine carries both a positive and a negative charge, therefore acidification of the sample is necessary to neutralize the acidic functional group for reproducible cation exchange at the amine functional group.

section 2 - EXTRACTION METHOD

SAMPLING PROCEDURE:

Target analytes show poor hydrolytic stability, particularly under alkaline conditions. Samples should be kept cool and dark as much as possible after collection. Blood samples are best preserved with fluoride and kept at a pH of 5.

SAMPLE PREPARATION:

A. Serum or Plasma:

To 1 mL of serum or plasma add internal standard(s)* and 4 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

B: Whole Blood:

See Appendix A for specimen treatment. Dilute 1 part resulting supernatant with 4 parts 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum.

2 mL 100 mM phosphate buffer (pH 6.0); draw through under vacuum.

NOTE: Use a low vacuum (≤ 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

* Suggested internal standard: bupivacaine

3. COLUMN RINSE

6 mL DI H₂O; draw through under vacuum.

3 mL 1 M acetic acid; draw through under vacuum.

Dry for 5 minutes under vacuum.

6 mL CH₃OH; draw through under vacuum.

4A.* ELUTE COCAINE AND BENZOYLECGONINE

2 mL CH₂Cl₂/IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

4B.* ELUTE COCAINE AND BENZOYLECGONINE

2 mL CH₃OH/NH₄OH (98/2); collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

5. CONCENTRATE

Evaporate to dryness at ≤ 40°C.

* Choose either 4A or 4B.

section 3 - ANALYSIS

Reconstitute with 100 µL methanol.

Inject 20 µL onto the HPLC system:

Wavelengths: 230, 255, 275 nm

Column: C-18 reverse phase

Flow rate: 1.5 mL/min.

Mobile Phase

0.025 M KH₂PO₄ - 500 mL

Acetonitrile - 125 mL

Butylamine - 12.5 mL

Adjust to pH 2.9 with H₃PO₄

Retention Times

Benzoylecgonine - 6.2 min.

Cocaine - 7.9 min.

Norcocaine - 9.1 min.

Bupivacaine (ISTD) - 10.1 min.

Cocaethylene - 12.3 min.

For method optimization tips, see p. 8

**EXTRACTION OF DRUGS OF ABUSE
USING BOND ELUT CERTIFY**



ANALYTE NAME/MATRIX	ANALYTICAL TECHNIQUE	PRODUCT/PART NUMBER USED
M2715 Cocaine And Benzoylecgonine in Urine	GC or GC/MS	130 mg Certify 1210-2051 or 1211-3050

section 1 - PRINCIPLE AND MECHANISMS

Basic drug extraction utilizing both non-polar and cation exchange mechanisms. At neutral pH, benzoylecgonine carries both a positive and a negative charge, therefore acidification of the sample is necessary to neutralize the acidic functional group for reproducible cation exchange at the amine functional group.

section 2 - EXTRACTION METHOD

SAMPLING PROCEDURE:

Target analytes show poor hydrolytic stability, particularly under alkaline conditions. Samples should be kept cool and dark as much as possible after collection. Adjust sample to a pH of 5 with dilute acetic acid (0.1 M).

SAMPLE PREPARATION:

To 5 mL of urine add internal standard(s)* and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum.

2 mL 100 mM phosphate buffer (pH 6.0); draw through under vacuum.

NOTE: Use a low vacuum (≤ 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

* Suggested internal standards for GC/MS: *d*₃-Cocaine, *d*₃-Benzoylecgonine. Suggested internal standards for GC/FID: a) analogues of benzoylecgonine (propylbenzoylecgonine), b) opiate alkaloids (levallorphan, nalorphine, ethylmorphine, codeine), c) misc. (*n*-tetracosane, tetraphenylethylene (FID only), butylanthraquinone)

3. COLUMN RINSE

- 6 mL DI H₂O; draw through under vacuum.
- 3 mL 1 M acetic acid; draw through under vacuum.
- Dry for 5 minutes under vacuum.
- 6 mL CH₃OH; draw through under vacuum.

4. ELUTE COCAINE AND BENZOYLECGONINE

- 2 mL CH₂Cl₂/IPA (80:20) containing 2% NH₄OH; collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

5. DRY ELUATE

- Evaporate to dryness at ≤ 40°C.

DERIVATIZATION:

Add 50 µL BSTFA (with 1% TMCS) and cap. Mix/vortex. React 20 minutes at 70°C. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA solution.

PFPA derivatization is also acceptable.

section 3 - ANALYSIS

Inject 1 to 3 µL sample (in BSTFA solution) into chromatograph. Monitor the following ions (Mass Selective Detection):

<u>Cocaine</u>	<u>d₃-Cocaine</u>	<u>TMS-BE</u>	<u>TMS- d₃-BE</u>
182**	185**	240**	243**
198	201	256	259
303	306	361	364

** Quantitation ion

For method optimization tips, see p. 8

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY

VARIAN



ANALYTE NAME/MATRIX	ANALYTICAL TECHNIQUE	PRODUCT/PART NUMBER USED
M2716 Cocaine And Metabolites From Meconium	GC or GC/MS	130 mg Certify 1210-2051 or 1211-3050

section 1 - PRINCIPLE AND MECHANISMS

Basic drug extraction utilizing both non-polar and cation exchange mechanisms. At neutral pH, benzoylecgonine carries both a positive and a negative charge, therefore acidification of the sample is necessary to neutralize the acidic functional group for reproducible cation exchange at the amine functional group.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

Vortex 0.5 - 1 g meconium and 2 mL of CH₃OH. Centrifuge and transfer the supernatant to a clean tube. To each tube add 3 mL 100 mM phosphate buffer (pH 6.0), internal standard* and vortex. Matrix must be more aqueous than organic for good retention to occur.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum.

2 mL 100 mM phosphate buffer (pH 6.0); draw through under vacuum.

NOTE: Use a low vacuum (\leq 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

3. COLUMN RINSE

6 mL DI H₂O; draw through under vacuum.

3 mL 1 M acetic acid; draw through under vacuum

Dry for 5 minutes under vacuum.

6 mL CH₃OH; draw through under vacuum.

* Suggested internal standards for GC/MS: *d*₃-Cocaine, *d*₃-Benzoylecgonine. Suggested internal standards for GC/FID: a) analogues of benzoylecgonine (propylbenzoylecgonine), b) opiate alkaloids (levallorphan, nalorphine, ethylmorphine, codeine), c) misc. (*n*-tetracosane, tetraphenylethylene (FID only), butylanthraquinone)

4. ELUTE ISOLATES

2 mL CH₂Cl₂/IPA (80:20) containing 2% NH₄OH; collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

5. EVAPORATE

Evaporate the elution solvent to dryness at $\leq 40^{\circ}\text{C}$.

DERIVATIZATION:

Add 50 μL BSTFA (with 1% TMCS) and cap. Mix/vortex. React 20 minutes at 70°C . Remove from heat source to cool.

NOTE: Do not evaporate BSTFA solution.

section 3 - ANALYSIS

Inject 1 - 3 μL sample (in BSTFA solution) onto the chromatograph.
Monitor the following ions (Mass Selective Detection)

<u>Cocaine</u>	<u><i>d</i>₃-Cocaine</u>	<u>TMS-BE</u>	<u>TMS- <i>d</i>₃-BE</u>
182**	185**	240**	243**
198	201	256	259
303	306	361	364

** Quantitation ion

For method optimization tips, see p. 8

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY

VARIAN 

ANALYTE NAME/MATRIX	ANALYTICAL TECHNIQUE	PRODUCT/PART NUMBER USED
M2717 Cocaine And Metabolites From Meconium	HPLC	130 mg Certify 1210-2051 or 1211-3050

section 1 - PRINCIPLE AND MECHANISMS

Basic drug extraction utilizing both non-polar and cation exchange mechanisms. At neutral pH, benzoylecgonine carries both a positive and a negative charge, therefore acidification of the sample is necessary to neutralize the acidic functional group for reproducible cation exchange at the amine functional group.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

Vortex 0.5 - 1 g meconium and 2 mL of CH₃OH. Centrifuge and transfer the supernatant to a clean tube. To each tube add 3 mL 100 mM phosphate buffer (pH 6.0), internal standard* and vortex. Matrix must be more aqueous than organic for good retention to occur.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum.

2 mL 100 mM phosphate buffer (pH 6.0); draw through under vacuum.

NOTE: Use a low vacuum (≤ 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

3. COLUMN RINSE

6 mL DI H₂O; draw through under vacuum.

3 mL 1 M acetic acid; draw through under vacuum

Dry for 5 minutes under vacuum.

6 mL CH₃OH; draw through under vacuum.

4. ELUTE ISOLATES

2 mL CH₂Cl₂/IPA (80:20) containing 2% NH₄OH; collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

5. EVAPORATE

Evaporate the elution solvent to dryness at $\leq 40^{\circ}\text{C}$.

* Suggested internal standard: bupivacaine

section 3 - ANALYSIS

Reconstitute with 100 µL methanol.

Inject 20 µL onto the HPLC system:

Wavelengths: 230, 255, 275 nm

Column: C-18 reverse phase

Flow rate: 1.5 mL/min.

Mobile Phase

0.025 M KH₂PO₄ - 500 mL

Acetonitrile - 125 mL

Butylamine - 12.5 mL

Adjust to pH 2.9 with H₃PO₄

Retention Times

Benzoyllecgonine - 6.2 min.

Cocaine - 7.9 min.

Norcocaine - 9.1 min.

Bupivacaine (ISTD) - 10.1 min.

Cocaethylene - 12.3 min.

For method optimization tips, see p. 8

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY

VARIAN 

ANALYTE NAME/MATRIX	ANALYTICAL TECHNIQUE	PRODUCT/PART NUMBER USED	
M2718 Fentanyl And Analogues In Urine	GC or GC/MS	130 mg Certify 1210-2051 or 1211-3050	

section 1 - PRINCIPLE AND MECHANISMS

Drug extraction using hydrophobic interactions for retention and ion exchange and secondary polar interactions to remove interferences.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

To 5 mL of sample add internal standard and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum.

2 mL DI H₂O; draw through under vacuum.

1 mL 100 mM phosphate buffer (pH 6.0); draw through under vacuum.

NOTE: Use a low vacuum (≤ 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

3. COLUMN RINSE

3 mL DI H₂O; draw through under vacuum.

2 mL 1.0 M acetic acid; draw through under vacuum.

3 mL CH₃OH; draw through under vacuum.

Dry column (5 minutes at ≥ 10 inches Hg).

4. ELUTE FENTANYLS

2 mL CH₂Cl₂/IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

5. CONCENTRATE

Evaporate to dryness at $\leq 40^\circ\text{C}$.

section 3 - ANALYSIS

Reconstitute with 50 μ L ethyl acetate.

Inject 1 to 3 μ L into chromatograph.

Monitor the following ions (Mass Selective Detection):

<u>Fentanyl</u>	<u><i>d</i>₅-Fentanyl</u>	<u>α-Methylfentanyl</u>	<u>p-Fluorofentanyl</u>	<u>3-Methylfentanyl</u>
245*	250*	259*	263*	259*
146	151	203	164	160
189	194	146	207	203
<u>Thienfentanyl</u>	<u>Sufentanil</u>	<u>Carfentanil</u>	<u>Lofentanil</u>	<u>Alfentanil</u>
245*	289*	303*	317*	289*
146	140	187	201	268
189			289	222

* Quantitation ion

For method optimization tips, see p. 8

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY



ANALYTE NAME/MATRIX M2719 Flunitrazepam in Urine	ANALYTICAL TECHNIQUE GC or GC/MS	PRODUCT/PART NUMBER USED 130 mg Certify 1210-2051 or 1211-3050	
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section 1 - PRINCIPLE AND MECHANISMS

Drug extraction using hydrophobic interactions to retain the drug and ion exchange and secondary polar interactions for sample clean up.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

To 5 mL of urine add internal standard(s) and 2 mL 0.1M phosphate buffer (adjusted to pH 6)

COLUMN PREPARATION/EXTRACTION:

1. CERTIFY EXTRACTION CARTRIDGE CONDITIONING:
 - 2 mL CH₃OH; draw through with vacuum.
 - 2 mL 0.1 M phosphate buffer (pH 6); draw through under vacuum.
2. SPECIMEN APPLICATION:
 - Load at 1 to 2 mL/minute.
3. COLUMN RINSE
 - 3 mL H₂O; draw through under vacuum.
 - 1 mL 1M CH₃COOH; draw through under vacuum.
 - Dry 15 mins at full vacuum.
 - 2 mL CH₃OH; draw through under vacuum.
4. ELUTE FLUNITRAZEPAM
 - 2 mL 2% NH₄OH in CH₂CL₂:IPA (8:2)
5. DRY ELUATE
 - Evaporate under N₂ to dryness.
 - Reconstitute in 50-100 µL EtOAc.
 - Inject into GC.

For method optimization tips, see p. 8

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY



ANALYTE NAME/MATRIX	ANALYTICAL TECHNIQUE	PRODUCT/PART NUMBER USED
M2720 Fluoxetine and Norfluoxetine in Serum, Plasma, or Whole Blood	GC or GC/MS	130 mg Certify 1210-2051 or 1211-3050

Section 1 - PRINCIPLE AND MECHANISMS

Basic drug extraction utilizing both non-polar and cation exchange mechanisms.

Section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

A. Serum or Plasma:

To 1 mL of serum or plasma add internal standard(s)* and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

B: Whole Blood:

See Appendix A for specimen treatment. Dilute 1 part resulting supernatant with 4 parts 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum.

2 mL DI H₂O; draw through under vacuum.

1 mL 100 mM phosphate buffer (pH 6.0); draw through under vacuum.

NOTE: Use a low vacuum (≤ 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

3. COLUMN RINSE

2 mL CH₃OH

2 mL acetonitrile

Dry column (5 min at > 10 " Hg).

2 mL hexane/ethyl acetate (50:50, v/v)

4. ELUTE FLUOXETINE, NORFLUOXETINE AND INTERNAL STANDARD

2 mL CH₂Cl₂/IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute.

5. DRY ELUATE

Evaporate to dryness at $\leq 40^{\circ}\text{C}$.

DERIVATIZATION:

Evaporate to 1 mL. Add 1 drop 0.3 N HCl in CH₃OH and vortex. Evaporate to dryness at room temperature under N₂. Add 100 μL 1% Et₃N in toluene; vortex. Add 20 μL PFPA. React at 90 $^{\circ}\text{C}$ for 30 minutes. Allow to cool to ambient temperature. Evaporate to dryness at ambient temperature. Reconstitute in 100 μL hexane.

Section 3 - ANALYSIS

Reconstitute with 200 μL ethyl acetate. Inject 2 μL .
Monitor the following ions (Mass Selective Detection):

<u>Norfluoxetine</u>	<u>Fluoxetine</u>	<u>Protriptyline</u>
117**	117	191**
176	190**	409
280	294	

* Suggested internal standard: Protriptyline

** Quantitation ion

For method optimization tips, see p. 8

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY

VARIAN 

ANALYTE NAME/MATRIX	ANALYTICAL TECHNIQUE	PRODUCT/PART NUMBER USED	
M2721 General Drug Screen from Urine or Plasma	GC or GC/FID	130 mg Certify 1210-2051 or 1211-3050	

section 1 - PRINCIPLE AND MECHANISMS

Basic, acidic and neutral drugs can be retained and selectively eluted using the ion exchange, polar, and non-polar interactions of the Certify mixed mode sorbant.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

To 2 mL of either urine or plasma add 6 mL of 0.1 M phosphate buffer (pH 6.0).
Mix/vortex.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum.

2 mL 0.1 M phosphate buffer (pH 6.0); draw through under vacuum.

NOTE: Use a low vacuum (2 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

3. COLUMN RINSE

1 mL DI H₂O; draw through under vacuum.

0.5 mL 0.01 M acetic acid; draw through under vacuum.

Dry column (4 minutes at 15 inches Hg).

50 µL CH₃OH (no vacuum).

Dry column (1 minute at 15 inches Hg).

4. ELUTE ACIDIC AND NEUTRAL DRUGS (FRACTION A)

4 mL acetone:chloroform (50/50); draw through slowly under low vacuum (1 inch Hg).

5. ELUTE BASIC DRUGS (FRACTION B)

2 mL EtOAc/NH₄OH (98/2); use no vacuum.

section 3 - ANALYSIS

Add 100 μL of a 200 $\mu\text{g}/\text{mL}$ Prazepam solution (internal standard). Mix/vortex. Evaporate each fraction to 100 μL at 40°C under N_2 . Inject 1 to 2 μL of each fraction into the gas chromatograph (GC).

For method optimization tips, see p. 8

section 4 - OTHER INFORMATION

CERTIFY GENERAL DRUG SCREEN ANALYSIS*

The following compounds have been extracted from urine and plasma samples using Certify extraction columns:

FRACTION (A): ACIDIC AND NEUTRAL DRUGS

Butalbital	Clonazepam	Methaqualone
Heptabarbital	Diazepam	Meprobamate
Hexobarbital	Lorazepam	
Metharbital	Nitrazepam	
Pentobarbital	Oxazepam	
Probarbital		
Secobarbital		

FRACTION (B): BASIC DRUGS

Amphetamine	Levallorphan	Procaine
Cocaine	Mepivacaine	Promethazine
Codeine	Methamphetamine	Trimipramine
Imipramine	Morphine**	

For more general information on these and other drugs of abuse, see John Wilson's "Abused Drugs, A Laboratory Pocket Guide"; AACC Press; Washington, D.C.: 1990.

* Adapted from Chen, X.-H. et al. *Journal of Forensic Sciences* **1992**, 37(1), 61-71.

** Requires 2 x 2 mL EtOAc/ NH_4OH (98/2) elution aliquots.

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY

VARIAN



ANALYTE NAME/MATRIX	ANALYTICAL TECHNIQUE	PRODUCT/PART NUMBER USED
M2722 Lysergic Acid Diethylamide (LSD) in Serum, Plasma, or Whole Blood	GC or GC/MS	130 mg Certify 1210-2051 or 1211-3050

section 1 - PRINCIPLE AND MECHANISMS

Basic drug extraction using non-polar and cation exchange mechanisms.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

A. Serum or Plasma:

To 1 mL of serum or plasma add internal standard(s)* and 4 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

B: Whole Blood:

See Appendix A for specimen treatment. Dilute 1 part resulting supernatant with 4 parts 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum.

2 mL DI H₂O; draw through under vacuum.

1 mL 100 mM phosphate buffer (pH 6.0); draw through under vacuum.

NOTE: Use a low vacuum (≤ 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

3. COLUMN RINSE

3 mL DI H₂O; draw through under vacuum.

1 mL 1.0 M acetic acid; draw through under vacuum.

3 mL CH₃OH; draw through under vacuum.

Dry column (5 minutes at ≥ 10 inches Hg).

4. ELUTE LSD

2 mL CH₂Cl₂/IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

5. DRY ELUATE

Evaporate to dryness at $\leq 40^{\circ}\text{C}$.

DERIVATIZATION:

Add 20 μL acetonitrile and 20 μL BSTFA (with 1% TMCS). Blanket with N_2 and cap. Mix/vortex. React 20 minutes at 70°C . Remove from heat source to cool.

NOTE: Do not evaporate BSTFA solution.

section 3 - ANALYSIS

Inject 1 to 3 μL sample (in BSTFA solution) into chromatograph.
Monitor the following ions (Mass Selective Detection):

<u>LSD</u>	<u>d_3-LSD</u>
395**	398**
293	296
268	271

* Suggested internal standard for GC/MS: d_3 -LSD

** Quantitation ion

For method optimization tips, see p. 8

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY



ANALYTE NAME/MATRIX	ANALYTICAL TECHNIQUE	PRODUCT/PART NUMBER USED
M2723 Lysergic Acid Diethylamide (LSD) in Urine	GC or GC/MS	130 mg Certify 1210-2051 or 1211-3050

section 1 - PRINCIPLE AND MECHANISMS

Basic drug extraction using non-polar and cation exchange mechanisms.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

To 5 mL of urine add internal standard* and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum.

2 mL DI H₂O; draw through under vacuum.

1 mL 100 mM phosphate buffer (pH 6.0); draw through under vacuum.

NOTE: Use a low vacuum (≤ 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

3. COLUMN RINSE

3 mL DI H₂O; draw through under vacuum.

1 mL 1.0 M acetic acid; draw through under vacuum.

3 mL CH₃OH; draw through under vacuum.

Dry column (5 minutes at ≥ 10 inches Hg).

4. ELUTE LSD

2 mL CH₂Cl₂/IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

5. DRY ELUATE

Evaporate to dryness at $\leq 40^{\circ}\text{C}$.

DERIVATIZATION:

Add 20 μL acetonitrile and 20 μL BSTFA (with 1% TMCS). Blanket with N_2 and cap. Mix/vortex. React 20 minutes at 70°C . Remove from heat source to cool.

NOTE: Do not evaporate BSTFA solution.

section 3 - ANALYSIS

Inject 1 to 3 μL sample (in BSTFA solution) into chromatograph.
Monitor the following ions (Mass Selective Detection):

<u>LSD</u>	<u><i>d</i>₃-LSD</u>
395**	398**
293	296
268	271

* Suggested internal standard for GC/MS: *d*₃-LSD

** Quantitation ion

For method optimization tips, see p. 8

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY

VARIAN 

ANALYTE NAME/MATRIX	ANALYTICAL TECHNIQUE	PRODUCT/PART NUMBER USED
M2724 Meperidine (Pethidine) in Urine	GC or GC/MS	130 mg Certify 1210-2051 or 1211-3050

section 1 - PRINCIPLE AND MECHANISMS

Basic drug extraction using non-polar and cation exchange mechanisms.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

To 5 mL of urine add internal standard* and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum.

2 mL DI H₂O; draw through under vacuum.

1 mL 100 mM phosphate buffer, pH 6.0; draw through under vacuum.

NOTE: Use a low vacuum (≤ 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

3. COLUMN RINSE

3 mL DI H₂O; draw through under vacuum.

1 mL 1.0 M acetic acid; draw through under vacuum.

3 mL CH₃OH; draw through under vacuum.

Dry column (5 minutes at ≥ 10 inches Hg).

4. ELUTE MEPERIDINE

2 mL CH₂Cl₂/IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

5. DRY ELUATE

Evaporate to dryness at $\leq 40^\circ\text{C}$. Remove immediately upon completion.

Reconstitute with 100 μL ethyl acetate.

section 3 - ANALYSIS

Inject 1 to 3 μL into chromatograph.

Monitor the following ions (Mass Selective Detection):

<u>Meperidine</u>	<u>Phenyltoloxamine</u>
247**	58**
218	
172	

* Suggested internal standard for GC/MS: Phenyltoloxamine

** Quantitation ion

For method optimization tips, see p. 8

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY

VARIAN 

ANALYTE NAME/MATRIX	ANALYTICAL TECHNIQUE	PRODUCT/PART NUMBER USED
M2725 Methadone in Urine	GC or GC/MS	130 mg Certify 1210-2051 or 1211-3050

section 1 - PRINCIPLE AND MECHANISMS

Basic drug extraction using non-polar and cation exchange mechanisms.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

To 5 mL of urine add internal standard(s)* and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum.

2 mL DI H₂O; draw through under vacuum.

1 mL 100 mM phosphate buffer (pH 6.0); draw through under vacuum.

NOTE: Use a low vacuum (≤ 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

3. COLUMN RINSE

3 mL DI H₂O; draw through under vacuum.

1 mL 1.0 M acetic acid; draw through under vacuum.

3 mL CH₃OH; draw through under vacuum.

Dry column (5 minutes at ≥ 10 inches Hg).

4. ELUTE METHADONE

2 mL 2% NH₄OH in ethyl acetate; collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

5. CONCENTRATE

Evaporate to dryness at $\leq 40^\circ\text{C}$.

Reconstitute with 100 μL ethyl acetate.

section 3 - ANALYSIS

Inject 1 to 3 μL into chromatograph.

Monitor the following ions (Mass Selective Detection):

<u>Methadone</u>	<u>Phenyltoloxamine</u>
72**	58**
91	
165	

* Suggested internal standards for GC/MS: d_3 -Methadone or Phenyltoloxamine

** Quantitation ion

For method optimization tips, see p. 8

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY

VARIAN 

ANALYTE NAME/MATRIX M2726 Methaqualone in Urine	ANALYTICAL TECHNIQUE GC or GC/MS	PRODUCT/PART NUMBER USED 130 mg Certify 1210-2051 or 1211-3050	
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section 1 - PRINCIPLE AND MECHANISMS

Drug extraction using hydrophobic interactions for retention and ion exchange and secondary polar interactions to remove interferences.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

To 5 mL of urine add internal standard* and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum.

2 mL DI H₂O; draw through under vacuum.

1 mL 100 mM phosphate buffer (pH 6.0); draw through under vacuum.

NOTE: Use a low vacuum (≤ 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

3. COLUMN RINSE

3 mL DI H₂O; draw through under vacuum.

Dry column (5 minutes at ≥ 10 inches).

2 mL hexane; draw through under vacuum.

4. ELUTE METHAQUALONE

2 mL hexane/ethyl acetate (50/50); collect eluate at ≤ 5 mL/minute.

5. DRY ELUATE

Evaporate to dryness at $\leq 40^\circ\text{C}$.

Reconstitute with 50 μL ethyl acetate.

section 3 - ANALYSIS

Inject 1 to 3 μL into chromatograph.

Monitor the following ions (Mass Selective Detection):

<u>Methaqualone</u>	<u>Hexobarbital</u>
235**	221**
250	157
233	156

* Suggested internal standard for GC/MS: Hexobarbital

** Quantitation ion

For method optimization tips, see p. 8

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY

VARIAN



ANALYTE NAME/MATRIX	ANALYTICAL TECHNIQUE	PRODUCT/PART NUMBER USED
M2727 6-Monoacetyl Morphine in Urine	GC or GC/MS	130 mg Certify 1210-2051 or 1211-3050

section 1 - PRINCIPLE AND MECHANISMS

Basic drug extraction using cation exchange and non-polar mechanisms. 6-monoacetyl morphine can confirm heroin use because it is a heroin metabolite but not a metabolite of codeine or morphine. Its presence can only be demonstrated soon after heroin intake as it is rapidly further metabolized

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

To 5 mL of urine add internal standard* and 2 mL of 10 mM phosphate buffer (pH 6.0). Mix/vortex. Adjust pH to 8.0-8.5 with KOH.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum.

2 mL 10 mM phosphate buffer (adjusted to pH 8.0-9.0 with 10 M KOH); draw through under vacuum.

NOTE: Use a low vacuum (≤ 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

3. COLUMN RINSE

2 mL DI H₂O; draw through under vacuum.

2 mL 10 mM phosphate buffer (adjusted to pH 4.0 with phosphoric acid); draw through under vacuum.

2 mL CH₃OH; draw through under vacuum.

Dry column (2 minutes at ≥ 10 inches Hg).

4. ELUTE 6-MONOACETYL MORPHINE

2 mL CH₂Cl₂/IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

5. DRY ELUATE

Evaporate to dryness at $\leq 40^\circ\text{C}$.

DERIVATIZATION:

Add 50 μ L BSTFA (with 1% TMCS) and cap. Mix/vortex. React 20 minutes at 70 $^{\circ}$ C. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA solution.

section 3 - ANALYSIS

Inject 1 to 3 μ L sample (in BSTFA solution) into chromatograph.

Monitor the following ions (Mass Selective Detection):

TMS-6-MAM: 399**, 340, 287 TMS-*d*₃-6-MAM: 402**, 343, 290

* Suggested internal standard for GC/MS: *d*₃-6-MAM

** Quantitation ion

For method optimization tips, see p. 8

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY

VARIAN 

ANALYTE NAME/MATRIX	ANALYTICAL TECHNIQUE	PRODUCT/PART NUMBER USED
M2728 Nicotine in Urine	GC or GC/MS	130 mg Certify 1210-2051 or 1211-3050

section 1 - PRINCIPLE AND MECHANISMS

Basic drug extraction using cation exchange and non-polar mechanisms.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

To 5 mL of urine add internal standard(s)* and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum.

2 mL 100 mM phosphate buffer (pH 6.0); draw through under vacuum.

NOTE: Use a low vacuum (≤ 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

3. COLUMN RINSE

1 mL 1.0 M acetic acid; draw through under vacuum and dry for 5 minutes under vacuum.

6 mL CH₃OH; draw through under vacuum.

Dry column (2 minutes at ≥ 10 inches Hg).

4. ELUTE NICOTINE

2 mL CH₂Cl₂/IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

5. DRY ELUATE

Evaporate to dryness at $\leq 40^\circ\text{C}$ under N₂. Remove immediately upon completion.

Reconstitute with 50 μL ethyl acetate.

For method optimization tips, see p. 8

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY

VARIAN 

ANALYTE NAME/MATRIX	ANALYTICAL TECHNIQUE	PRODUCT/PART NUMBER USED
M2729 Opiates (Free/Unbound) in Serum, Plasma, or Whole Blood	GC or GC/MS	130 mg Certify 1210-2051 or 1211-3050

section 1 - PRINCIPLE AND MECHANISMS

Basic drug extraction using cation exchange and non-polar mechanisms.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

A. Serum or Plasma:

To 1 mL of serum or plasma add internal standard(s)* and 4 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Adjust pH to 8.0-8.5 with 10 M KOH.

B: Whole Blood:

See Appendix A for specimen treatment. Dilute 1 part resulting supernatant with 4 parts 100 mM phosphate buffer (pH 6.0). Mix/vortex. Adjust pH to 8.0-8.5 with 10 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum.

2 mL 100 mM phosphate buffer (adjusted to pH 8.0-9.0 with 10 M KOH); draw through under vacuum.

NOTE: Use a low vacuum (≤ 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

3. COLUMN RINSE

2 mL DI H₂O; draw through under vacuum.

2 mL 100 mM acetate buffer (pH 4.0); draw through under vacuum.

2 mL CH₃OH; draw through under vacuum.

Dry column (2 minutes at ≥ 10 inches Hg).

4. ELUTE OPIATES

2 mL CH₃OH/NH₄OH (98/2); collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

5. DRY ELUATE

Evaporate to dryness at $\leq 40^{\circ}\text{C}$.

DERIVATIZATION:

Add 50 μL BSTFA (with 1% TMCS) and cap. Mix/vortex. React 20 minutes at 70 $^{\circ}\text{C}$. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA solution.

section 3 - ANALYSIS

Inject 1 to 2 μL sample (in BSTFA solution) into chromatograph.

Monitor the following ions (Mass Selective Detection):

<u>TMS-Codeine</u>	<u>TMS-d_3-Codeine</u>	<u>TMS-Morphine</u>	<u>TMS-d_3-Morphine</u>
371**	374**	429**	432**
234	237	287	290
343	346	324	327

* Suggested internal standards for GC/MS: d_3 -Codeine, d_3 -Morphine. Suggested internal standards for other GC: nalorphine, alkanes such as tetracosane or docosane

** Quantitation ion

For method optimization tips, see p. 8

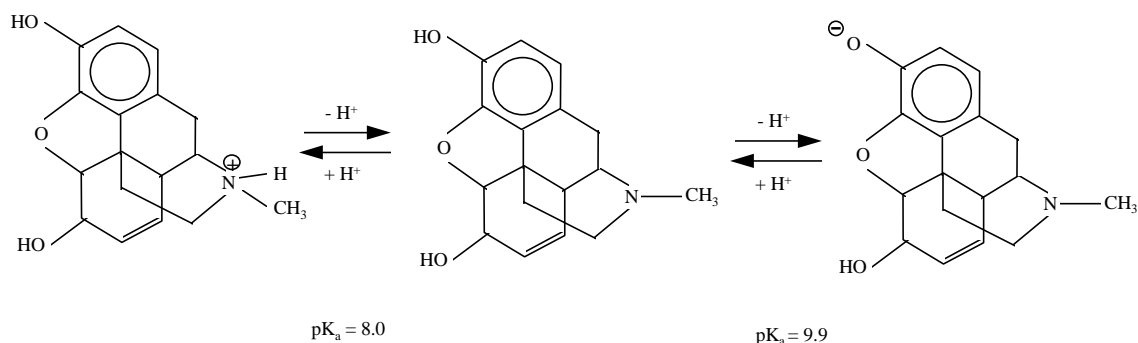
EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY



ANALYTE NAME/MATRIX	ANALYTICAL TECHNIQUE	PRODUCT/PART NUMBER USED
M2730 Opiates in Urine	GC or GC/MS	130 mg Certify 1210-2051 or 1211-3050

section 1 - PRINCIPLE AND MECHANISMS

Basic drug extraction using cation exchange and non-polar mechanisms. Morphine can behave as either an acid or a base depending on the sample pH. Therefore, careful monitoring of pH in sample preparation is crucial for reproducible recoveries. (See also section 4).



section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

Choose A or B.

A. ENZYMATIC HYDROLYSIS OF GLUCURONIDE:

To 5 mL of urine add internal standard(s)* and 2 mL of β -glucuronidase. β -glucuronidase: 5,000 F units/mL *Patella Vulgata* in 1.0 M acetate buffer (pH 5.0). Mix/vortex. Hydrolyze for 3 hours at 65 °C. Cool before proceeding. Adjust sample pH to 8.0-8.5 with 10 M KOH.

B. ACID HYDROLYSIS OF GLUCURONIDE (see also section 4):

To 5 mL of urine add internal standard(s)* and 1 mL concentrated HCl. Mix/vortex. Immerse in a hot water bath for at least 30 minutes at 100°C. Cool before proceeding. Add 2 mL 0.1 M potassium phosphate buffer, pH 6. Mix/vortex. Adjust sample pH to between 8.0-8.5 with 10.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum.
2 mL 100 mM phosphate buffer (adjusted to pH 8.0-9.0 with 10 M KOH); draw through under vacuum.

NOTE: Use a low vacuum (≤ 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

3. COLUMN RINSE

2 mL DI H₂O; draw through under vacuum.
2 mL 100 mM acetate buffer (pH 4.0); draw through under vacuum.
2 mL CH₃OH; draw through under vacuum.
Dry column (2 minutes at ≥ 10 inches Hg).

5. ELUTE OPIATES

2 mL CH₃OH/NH₄OH (98/2); collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

6. DRY ELUATE

Evaporate to dryness at $\leq 40^\circ\text{C}$.

DERIVATIZATION:

Add 50 μL BSTFA (with 1% TMCS) and cap. Mix/vortex. React 20 minutes at 70 $^\circ\text{C}$. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA solution.

Other acceptable derivatization reagents: MBTFA, PFPA, TFAA

section 3 - ANALYSIS

Inject 1 to 2 μL sample (in BSTFA solution) into chromatograph.

Monitor the following ions (Mass Selective Detection):

<u>TMS-Codeine</u>	<u>TMS-<i>d</i>₃-Codeine</u>	<u>TMS-Morphine</u>	<u>TMS-<i>d</i>₃-Morphine</u>
371**	374**	429**	432**
234	237	287	290
343	346	324	327

* Suggested internal standards for GC/MS: *d*₃-Codeine, *d*₃-Morphine. Suggested internal standards for other GC: nalorphine, alkanes such as tetracosane or docosane

** Quantitation ion

For method optimization tips, see p. 8

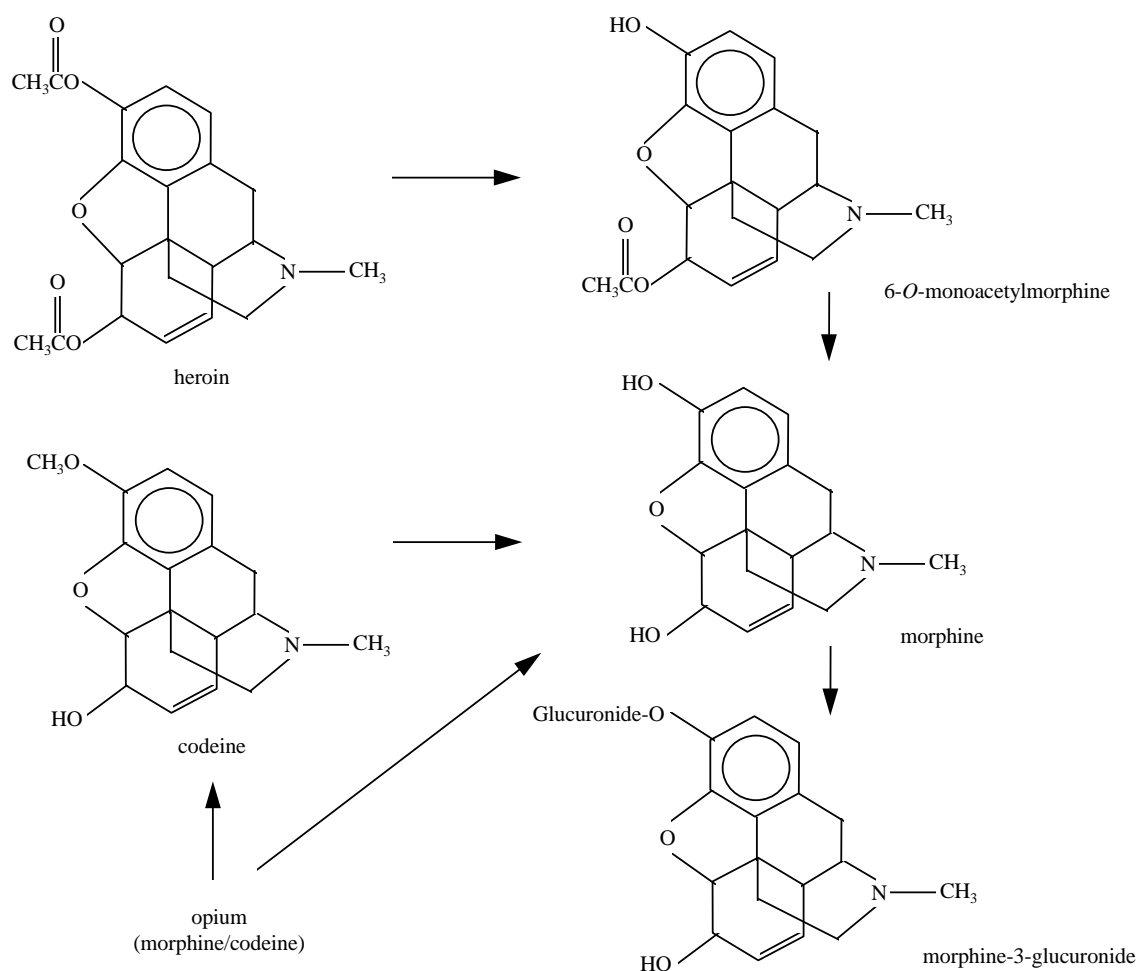
section 4 - ADDITIONAL TIPS ON ACID HYDROLYSIS

Successful Certify extraction after acid hydrolysis relies heavily on careful pH adjustment up to pH 8.5. It is very important that the pH never exceeds pH 8.5. (If it does, significant decrease in morphine recovery with little change in codeine recovery will be observed.) An excellent way to achieve proper pH is to titrate the working solution of 10 M KOH on blank samples to determine the volume of KOH required to adjust 1.00 mL of concentrated HCl to a pH of 8.5:

1. Add 2.0 mL DI water followed by 1.0 mL conc. HCl to a 16 x 100 mm test tube
2. Add 2.0 mL 1 M potassium phosphate buffer, pH 6.
3. Add 0.8 mL 10 M KOH and measure pH with a pH meter.
4. Add 100 μ L 10 M KOH and measure pH.
5. Continue to add 100 μ L aliquots of KOH until pH 8.5 is reached.
6. Note total volume used.

section 5 - METABOLISM AND EXCRETION

- Heroin is rapidly metabolized to 6-monoacetyl morphine, followed by slower hydrolysis to morphine, morphine-3-glucuronide.
- Major metabolites in urine until 20-40 h after intravenous administration are: morphine-3-glucuronide (38.2% of dose), free morphine (4.2%), 6-MAM (1.3%), and unchanged heroin (0.1%). Other morphine glucuronides and normorphine may be found as minor metabolites.
- Codeine has often been found in the urine of heroin users, but is not a metabolite of heroin (see below). It arises from the deacetylation of acetylcodeine, an impurity found in illicit heroin.



EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY

VARIAN 

ANALYTE NAME/MATRIX	ANALYTICAL TECHNIQUE	PRODUCT/PART NUMBER USED
M2731 Phencyclidine (PCP) in Urine	GC or GC/MS	130 mg Certify 1210-2051 or 1211-3050

section 1 - PRINCIPLE AND MECHANISMS

Basic drug extraction using cation exchange and non-polar mechanisms.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

To 5 mL of urine add internal standard(s)* and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum.

2 mL 100 mM phosphate buffer (pH 6.0); draw through under vacuum.

NOTE: Use a low vacuum (≤ 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

3. COLUMN RINSE

1 mL 1.0 M acetic acid; draw through under vacuum and dry for 5 minutes under vacuum.

6 mL CH₃OH; draw through under vacuum.

Dry column (2 minutes at ≥ 10 inches Hg).

4. ELUTE PHENCYCLIDINE

2 mL CH₂Cl₂/IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

5. DRY ELUATE

Evaporate to dryness at $\leq 40^\circ\text{C}$ under N₂. Remove immediately upon completion.

Reconstitute with 50 μL ethyl acetate.

section 3 - ANALYSIS

Inject 1 to 2 μL into chromatograph.

Monitor the following ions (Mass Selective Detection):

<u>Phencyclidine</u>	<u><i>d</i>₅-Phencyclidine</u>
200**	205**
91	96
242	247

* Suggested internal standard for GC/MS: *d*₅-Phencyclidine

* Suggested internal standard (non-GC/MS): Ketamine

** Quantitation ion

For method optimization tips, see p. 8

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY

VARIAN 

ANALYTE NAME/MATRIX M2732 Propoxyphene in Urine	ANALYTICAL TECHNIQUE GC or GC/MS	PRODUCT/PART NUMBER USED 130 mg Certify 1210-2051 or 1211-3050	
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section 1 - PRINCIPLE AND MECHANISMS

Basic drug extraction using cation exchange and non-polar mechanisms.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

To 5 mL urine add internal standard(s)* and 2 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum.

2 mL DI H₂O; draw through under vacuum.

1 mL 100 mM phosphate buffer, (pH 6.0); draw through under vacuum.

NOTE: Use a low vacuum (≤ 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

3. COLUMN RINSE

3 mL DI H₂O; draw through under vacuum.

1 mL 1.0 M acetic acid; draw through under vacuum.

3 mL CH₃OH; draw through under vacuum.

Dry column (5 minutes at ≥ 10 inches Hg).

4. ELUTE PROPOXYPHENE

2 mL CH₂Cl₂/IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent daily.

5. CONCENTRATE

Evaporate to dryness at $\leq 40^\circ\text{C}$.

Reconstitute with 100 μL ethyl acetate.

section 3 - ANALYSIS

Inject 1 to 3 µL into chromatograph.

Monitor the following ions (Mass Selective Detection):

<u>Propoxyphene</u>	<u>Phenyltoloxamine</u>
58**	58**
115	
208	

* Suggested internal standard for GC/MS: *d*₅-Propoxyphene or Phenyltoloxamine

** Quantitation ion

For method optimization tips, see p. 8

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY

VARIAN



ANALYTE NAME/MATRIX	ANALYTICAL TECHNIQUE	PRODUCT/PART NUMBER USED	
M2733 Sertraline and Desmethylsertraline in Serum, Plasma, or Whole Blood	HPLC	130 mg Certify 1210-2051 or 1211-3050	

section 1 - PRINCIPLE AND MECHANISMS

Basic drug extraction using cation exchange and non-polar mechanisms.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

A. Serum or Plasma:

To 1 mL of serum or plasma add internal standard(s)* and 4 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

B: Whole Blood:

See Appendix A for specimen treatment. Dilute 1 part resulting supernatant with 4 parts 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum.

2 mL DI H₂O; draw through under vacuum.

1 mL 100 mM phosphate buffer (pH 6.0); draw through under vacuum.

NOTE: Use a low vacuum (≤ 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

3. COLUMN RINSE

3 mL DI H₂O; draw through under vacuum.

1 mL 1.0 M acetic acid; draw through under vacuum.

3 mL CH₃OH; draw through under vacuum.

Dry column (5 minutes at ≥ 10 inches Hg).

4. ELUTE ISOLATES

2 mL CH₂Cl₂/IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent fresh daily.

5. DRY ELUATE

Evaporate to dryness at $\leq 40^{\circ}\text{C}$.

section 3 - ANALYSIS

Reconstitute with 200 μL acetonitrile/DI H_2O (25/75). Mix/vortex vigorously for 30 seconds. Inject 100 μL into chromatograph at wavelength 235 nm. Mobile phase (from literature) = 0.25 M K_2HPO_4 (pH 2.7) containing 30% CH_3CN . Flow rate 2 mL/minute.

For method optimization tips, see p. 8

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY

VARIAN



ANALYTE NAME/MATRIX	ANALYTICAL TECHNIQUE	PRODUCT/PART NUMBER USED
M2734 THC and Carboxy-THC in Serum, Plasma, or Whole Blood	GC or GC/MS	130 mg Certify 1210-2051 or 1211-3050

section 1 - PRINCIPLE AND MECHANISMS

Drug extraction using hydrophobic interactions for retention and ion exchange and secondary polar interactions to remove interferences.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

Choose A, B, or C

Serum, Plasma, or Whole Blood:

- A. To 1 mL sample add internal standard(s)* and 1 mL acetonitrile. Mix/vortex. Centrifuge and transfer supernatant to a clean test tube. To the supernatant add 5 mL 100 mM acetic acid.
- B. To 1 mL sample add internal standard(s)* and 2 mL 30% acetonitrile. Mix/vortex. Centrifuge and transfer supernatant to a clean test tube. To the supernatant add 5 mL 100 mM acetate buffer (pH 4.0).

Serum or Plasma:

- C. To 1 mL sample add internal standard(s)* and 5 mL 100 mM acetate buffer (pH 4.0). Mix/vortex and centrifuge.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum. 2 mL 50 mM phosphoric acid.

NOTE: Use a low vacuum (≤ 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

3. COLUMN RINSE

9 mL 50 mM phosphoric acid; draw through under vacuum.

3 mL 50 mM phosphoric acid/CH₃OH (80/20); draw through under vacuum.

Dry column (10 minutes at ≥ 10 inches Hg).

200 μ L hexane; draw through under vacuum.

4. ELUTE THC AND CARBOXY-THC

1 mL hexane/ethyl acetate (80/20); collect eluate at ≤ 5 mL/minute.

5. DRY ELUATE

Evaporate to dryness at $\leq 40^\circ\text{C}$.

DERIVATIZATION

Add 50 μL BSTFA (with 1% TMCS) and cap. Mix/vortex. React 20 minutes at 70°C . Remove from heat source to cool.

NOTE: Do not evaporate BSTFA solution.

Other acceptable derivatization reagents: BSA, MSTFA, MTBSTFA, PFBBR, TFAA, TMPAH, TMSI

section 3 - ANALYSIS

Inject 1 to 3 μL sample (in BSTFA solution) into chromatograph.

Monitor the following ions (Mass Selective Detection):

<u>THC</u>	<u>d_3-THC</u>	<u>Carboxy-Δ^9-THC</u>	<u>d_3-Carboxy-Δ^9-THC</u>
303**	306**	371**	374**
315	318	473	476
386	389	488	491

* Suggested internal standards for GC/MS: d_3 -THC and d_3 -Carboxy- Δ^9 -THC

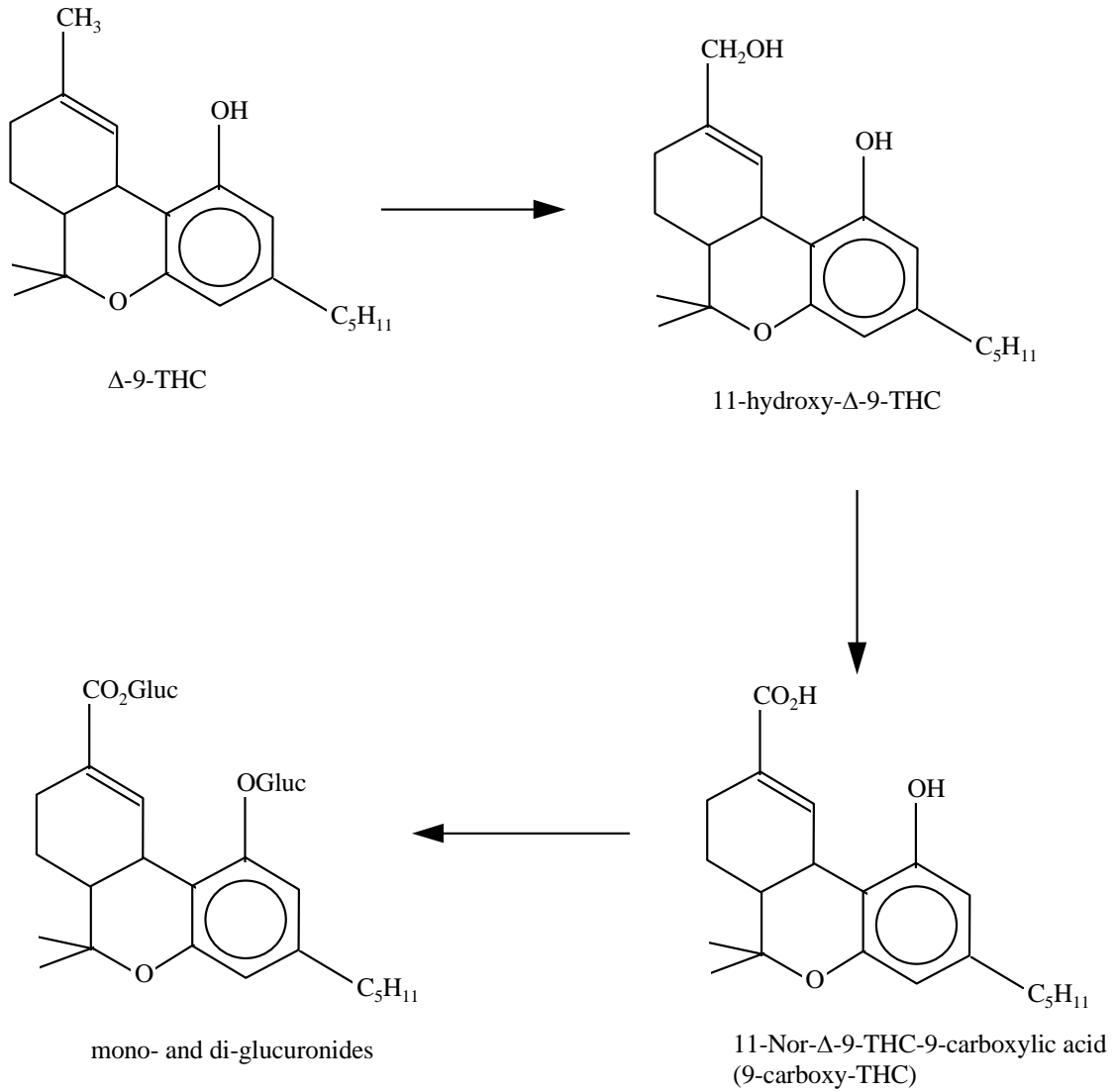
* Suggested internal standards for other GC: cannabinol, oxyphenbutazone, ketoprofen

** Quantitation ion

For method optimization tips, see p. 8

section 4 - METABOLISM AND EXCRETION

- THC is extensively metabolized – less than 1% of unchanged THC is recovered in the urine
- Within 72 h after smoking, approximately 50% of the inhaled THC will be excreted as the metabolite, and the remaining 50% distributed throughout fatty tissue in the body.
- Major THC metabolite is 9-carboxy-THC, which is converted to glucuronide conjugates
- 20 other THC metabolites have been identified.
- In occasional user, metabolite is detectable in urine for 1-3 days, whereas in chronic users it is detectable for a week or more.



EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY

VARIAN 

ANALYTE NAME/MATRIX M2735 THC and Carboxy-THC in Urine	ANALYTICAL TECHNIQUE GC or GC/MS	PRODUCT/PART NUMBER USED 130 mg Certify 1210-2051 or 1211-3050	
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section 1 - PRINCIPLE AND MECHANISMS

Drug extraction using hydrophobic interactions for retention and ion exchange and secondary polar interactions to remove interferences.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

BASE HYDROLYSIS OF GLUCURONIDE

To 3 mL of urine add internal standards* and 300 μ L of 10 M KOH. Mix/vortex. Hydrolyze for 15 minutes at 60°C. Cool before proceeding. Add 400 μ L glacial acetic acid and 3 mL 50 mM phosphoric acid. Mix/vortex. Sample pH should be between 4.0-5.0. If not, adjust the pH to this range.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum. 2 mL 50 mM phosphoric acid.

NOTE: Use a low vacuum (\leq 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

3. COLUMN RINSE

3 mL 50 mM phosphoric acid; draw through under vacuum.

3 mL 50 mM phosphoric acid/CH₃OH (80/20); draw through under vacuum.

Dry column (3 minutes at \geq 10 inches Hg).

200 μ L hexane; draw through under vacuum.

4. ELUTE THC AND CARBOXY-THC

2 mL hexane/ethyl acetate (80/20); collect eluate at \leq 5 mL/minute.

5. DRY ELUATE

Evaporate to dryness at \leq 40°C.

DERIVATIZATION:

Add 50 µL BSTFA (with 1% TMCS) and cap. Mix/vortex. React 20 minutes at 90 °C. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA.

Other acceptable derivatization reagents: BSA, MSTFA, MTBSTFA, PFBBBr, TFAA, TMPAH, TMSI

section 3 - ANALYSIS

Inject 1 to 3 µL sample (in BSTFA solution) into chromatograph.
Monitor the following ions (Mass Selective Detection):

<u>THC</u>	<u><i>d</i>₃-THC</u>	<u>Carboxy-Δ^9-THC</u>	<u><i>d</i>₃-Carboxy-Δ^9-THC</u>
303**	306**	371**	374**
315	318	473	476
386	389	488	491

* Suggested internal standards for GC/MS: *d*₃-THC and *d*₃-Carboxy- Δ^9 -THC

* Suggested internal standards for other GC: cannabinol, oxyphenbutazone, ketoprofen

** Quantitation ion

For method optimization tips, see p. 8

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY

VARIAN



ANALYTE NAME/MATRIX	ANALYTICAL TECHNIQUE	PRODUCT/PART NUMBER USED
M2736 Tricyclic Antidepressants in Serum, Plasma, or Whole Blood	GC or GC/MS	130 mg Certify 1210-2051 or 1211-3050

section 1 - PRINCIPLE AND MECHANISMS

Drug extraction using hydrophobic interactions for retention and ion exchange and secondary polar interactions to remove interferences.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

A. Serum or Plasma:

To 1 mL of serum or plasma add internal standard(s)* and 4 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

B: Whole Blood:

See Appendix A for specimen treatment. Dilute 1 part resulting supernatant with 4 parts 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum.

2 mL DI H₂O; draw through under vacuum.

1 mL 100 mM phosphate buffer (pH 6.0); draw through under vacuum.

NOTE: Use a low vacuum (≤ 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

3. COLUMN RINSE

3 mL DI H₂O; draw through under vacuum.

1 mL 1.0 M acetic acid; draw through under vacuum.

3 mL CH₃OH; draw through under vacuum.

Dry column (5 minutes at ≥ 10 inches Hg).

4. ELUTE TRICYCLIC ANTIDEPRESSANTS

2 mL CH₂Cl₂/IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent fresh daily.

5. DRY ELUATE

Evaporate to dryness at ≤ 40°C.

DERIVATIZATION:

Reconstitute with 50 µL ethyl acetate. Add 50 µL of pentafluoropropionic anhydride (PFPA) to derivatize. Blanket with N₂ and cap. React 20 minutes at 70 °C. Evaporate to dryness at ≤ 40°C. Reconstitute with 100 µL ethyl acetate.

Underivatized analyte can also be analyzed.

section 3 - ANALYSIS

A. UNDERIVATIZED ANALYSIS

Reconstitute with 100 µL CH₃OH. Inject 1-3 µL onto GC/NPD or GC/MS.

B. DERIVATIZED (PFPA) ANALYSIS

Inject 1 to 3 µL onto GC/NPD or GC/MS.

- Suggested internal standards: Clomipramine or Protriptyline

For method optimization tips, see p. 8

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY

VARIAN 

ANALYTE NAME/MATRIX	ANALYTICAL TECHNIQUE	PRODUCT/PART NUMBER USED	
M2737 Tricyclic Antidepressants in Serum, Plasma, or Whole Blood	HPLC	130 mg Certify 1210-2051 or 1211-3050	

section 1 - PRINCIPLE AND MECHANISMS

Drug extraction using hydrophobic interactions for retention and ion exchange and secondary polar interactions to remove interferences.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

A. Serum or Plasma:

To 1 mL of serum or plasma add internal standard(s)* and 4 mL of 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

B: Whole Blood:

See Appendix A for specimen treatment. Dilute 1 part resulting supernatant with 4 parts 100 mM phosphate buffer (pH 6.0). Mix/vortex. Sample pH should be 6.0 ± 0.5 . Adjust pH with 1.0 M KOH.

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY PREPARATION

2 mL CH₃OH; draw through under vacuum.

2 mL DI H₂O; draw through under vacuum.

1 mL 100 mM phosphate buffer (pH 6.0); draw through under vacuum.

NOTE: Use a low vacuum (≤ 3 inches Hg) to prevent drying of sorbent.

2. SPECIMEN APPLICATION

Load at 1 to 2 mL/minute.

3. COLUMN RINSE

3 mL DI H₂O; draw through under vacuum.

1 mL 1.0 M acetic acid; draw through under vacuum.

3 mL CH₃OH; draw through under vacuum.

Dry column (5 minutes at ≥ 10 inches Hg).

4. ELUTE TRICYCLIC ANTIDEPRESSANTS

2 mL CH₂Cl₂/IPA/NH₄OH (78/20/2); collect eluate at 1 to 2 mL/minute.

NOTE: Prepare elution solvent fresh daily.

5. DRY ELUATE

Evaporate to dryness at ≤ 40°C.

section 3 - ANALYSIS

Reconstitute with 200 µL acetonitrile/DI H₂O (25/75). Mix/vortex vigorously for 30 seconds. Inject 100 µL into chromatograph.

HPLC Conditions:

Column:	endcapped propylcyano, 4.6 mm (i.d.) x 15.0 cm, 5 µm
Temperature:	30 °C
Mobile phase:	CH ₃ CN/phosphate buffer/CH ₃ OH (60/25/15)
Phosphate buffer:	10 mM K ₂ HPO ₄ adjusted to pH 7.0 with 10 mM H ₃ PO ₄
Flow rate:	1.75 mL/minute

- Suggested internal standards: trimipramine and protriptyline

For method optimization tips, see p. 8

***EXTRACTION OF DRUGS OF ABUSE
USING BOND ELUT CERTIFY II***



ANALYTE NAME/MATRIX M2738 Barbiturates in Urine	ANALYTICAL TECHNIQUE GC or GC/MS	PRODUCT/PART NUMBER USED 130 mg Certify II 1210-2080 or 1211-3051
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section 1 - PRINCIPLE AND MECHANISMS

Acidic drug extraction using a non-polar mechanism for retention. Applicable drugs include amobarbital, butabarbital, pentobarbital, phenobarbital, secobarbital, and methaqualone.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

Add internal standard(s)* and 2 mL 100 mM sodium acetate buffer (pH 7.0).

COLUMN PREPARATION/EXTRACTION:

1. CERTIFY EXTRACTION CARTRIDGE CONDITIONING:
 - 1 mL CH₃OH; draw through with vacuum.
 - 1 mL 10 mM sodium acetate buffer (pH 7.0); draw through under vacuum.
2. SPECIMEN APPLICATION:
 - Load at 1 to 2 mL/minute.
3. COLUMN RINSE
 - 1 mL 100 mM sodium acetate buffer (pH 7.0); draw through under vacuum
 - Dry 5 mins at full vacuum.
 - 2 mL hexane/ethyl acetate (95:5)
4. ELUTE ANALYTES
 - 2 mL hexane/ethyl acetate (75:25)
5. DRY EXTRACT
 - Evaporate solvent at room temperature under a slow stream of nitrogen

For method optimization tips, see p. 8

section 3 - ANALYSIS

Reconstitute in 100 μ L of ethyl acetate.

Inject 1 to 2 μ L into chromatograph.

Monitor the following ions (Mass Selective Detection):

<u>Amobarbital</u>	<u>Butabarbital</u>	<u>Butalbital</u>	<u>Hexobarbital</u>
156**	156**	168**	221**
141	141	153	157
157	157	141	156
<u>Pentobarbital</u>	<u>Phenobarbital</u>	<u>Secobarbital</u>	
156**	204**	168**	
141	117	153	
157	232	195	

* Suggested internal standard for GC/MS: Hexobarbital

** Quantitation ion

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY II

VARIAN



ANALYTE NAME/MATRIX	ANALYTICAL TECHNIQUE	PRODUCT/PART NUMBER USED	
M2739 Non-Steroidal Anti-Inflammatory Drugs In Urine	HPLC	130 mg Certify II 1210-2080 or 1211-3051	

section 1 - PRINCIPLE AND MECHANISMS

Acidic drug extraction using a non-polar mechanism for retention. Applicable drugs include salicylic acid, naproxen, ibuprofen, indomethacin. If salicylic acid is not assayed, anion exchange can also be employed as a retention mechanism.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

Hydrolyze 2 mL of urine with 200 μ L 10 M KOH for 15 mins @ 60 °C. Cool; adjust pH to 2.0 with conc. HCl. Add internal standard(s) and 2 mL 10 mM sodium acetate buffer (pH 2.0).

COLUMN PREPARATION/EXTRACTION:

1. CERTIFY EXTRACTION CARTRIDGE CONDITIONING:
 - 2 mL CH₃OH; draw through with vacuum.
 - 2 mL 10 mM sodium acetate buffer (pH 2); draw through under vacuum.
2. SPECIMEN APPLICATION:
 - Load at 1 to 2 mL/minute.
3. COLUMN RINSE
 - 2 mL 10 mM sodium acetate buffer (pH 2); draw through under vacuum
 - 2 mL 10% aqueous acetic acid.
 - Dry 5 mins at full vacuum.
4. ELUTE ANALYTES
 - 2 mL 100 mM phosphoric acid/acetonitrile (1:1).

For method optimization tips, see p. 8

section 3 - ANALYSIS

Inject 100 μ L of the extract into the HPLC column for analysis.

HPLC conditions:

Column: C8 4.5 μ m, 4.6 x 150 mm

Mobile phase: 7 mM phosphoric acid:acetonitrile (50:50) (v/v)

Flow rate: $T_0 - T_5 = 1.0$ mL/min; $T_6 - T_{10} = 1.5$ mL/min.

EXTRACTION OF DRUGS OF ABUSE FROM URINE USING BOND ELUT CERTIFY II

VARIAN



ANALYTE NAME/MATRIX	ANALYTICAL TECHNIQUE	PRODUCT/PART NUMBER USED
M2740 11-Nor-Δ-9-Tetrahydrocannabinol-9-Carboxylic Acid (THC Metabolite) in Urine	GC or GC/MS	130 mg Certify II 1210-2080 or 1211-3051

section 1 - PRINCIPLE AND MECHANISMS

Acidic drug extraction using non-polar and anion exchange mechanisms.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

Hydrolyze 3 mL of urine with 300 μ L 10 M KOH for 15 mins @ 60 $^{\circ}$ C. Cool; add 165 μ L glacial acetic acid; pH should be between 4.5-6.5. Add internal standard(s)* and 2 mL 0.1 M sodium acetate solution, adjusted to pH 7, and containing 5% (vol) methanol.

COLUMN PREPARATION/EXTRACTION:

1. CERTIFY EXTRACTION CARTRIDGE CONDITIONING:
 - 1 mL CH₃OH; draw through with vacuum.
 - 1 mL 0.1 M sodium acetate solution, adjusted to pH 7 with HCl, and containing 5% (vol) methanol; draw through under vacuum.
2. SPECIMEN APPLICATION:
 - Load at 1 to 2 mL/minute.
3. COLUMN RINSE
 - 2 mL 50% methanol; draw through under vacuum.
 - Dry 1 min at full vacuum.
 - Important: Do not exceed drying time.***
4. ELUTE THC METABOLITE
 - 2 mL hexane:ethyl acetate (75:25) with 1% glacial acetic acid.

DERIVATIZATION:

Add 50 μ L BSTFA (with 1% TMCS) and cap. Mix/vortex. React 20 minutes at 90 $^{\circ}$ C. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA.

Other acceptable derivatization reagents: BSA, MSTFA, MTBSTFA, PFBBR, TFAA, TMPAH, TMSI

section 3 - ANALYSIS

Inject 1 to 3 μL sample (in BSTFA solution) into chromatograph.

Monitor the following ions (Mass Selective Detection):

<u>THC</u>	<u>d_3-THC</u>	<u>Carboxy-Δ^9-THC</u>	<u>d_3-Carboxy-Δ^9-THC</u>
303**	306**	371**	374**
315	318	473	476
386	389	488	491

* Suggested internal standards for GC/MS: d_3 -THC and d_3 -Carboxy- Δ^9 -THC

* Suggested internal standards for other GC: cannabinal, oxyphenbutazone, ketoprofen

** Quantitation ion

For method optimization tips, see p. 8

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY II

VARIAN 

ANALYTE NAME/MATRIX M2741 Cannabinoids in Whole Blood	ANALYTICAL TECHNIQUE GC or GC/MS	PRODUCT/PART NUMBER USED 200 mg Certify II 1210-2080 or 1211-3051	
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section 1 - PRINCIPLE AND MECHANISMS

Acidic and neutral drug extraction using hydrophobic and anion exchange mechanisms.

section 2 - EXTRACTION METHOD

SAMPLE PREPARATION:

1. Add 0.8 mL 0.1 M K₂HPO₄ (pH adjusted to 7.0), to 1.0 mL blood.
2. Add 200 µL β-glucuronidase to the mixture, cap, and vortex.
3. Incubate at 37 °C for 16 to 18 h. Add internal standards.*
4. Add 4 mL acetonitrile dropwise while vortexing
5. Centrifuge; transfer supernatant to clean test tube.
6. Add 16 mL 0.1 M K₂HPO₄, pH 7.0/CH₃OH (95:5) (v/v)

COLUMN PREPARATION/EXTRACTION:

1. BOND ELUT CERTIFY II PREPARATION:
2 mL CH₃OH; draw through under vacuum.
2 mL 0.1 M K₂HPO₄, pH 7.0/CH₃OH (95:5) (v/v); draw through under vacuum.
2. SPECIMEN APPLICATION
Load at 1 to 2 mL/min
3. COLUMN RINSE
1 mL 0.1 M K₂HPO₄, pH 7.0/CH₃OH (95:5) (v/v); draw through under vacuum and allow to dry for 10 seconds. Stop vacuum immediately.
Add 100 µL acetone; draw through under vacuum and allow to dry for 2 minutes under vacuum.
NOTE: Do not exceed 2 minute drying time.
4. ELUTE THC:
Elute 2 x 2 mL hexane/ethyl acetate (95:5) (v/v); collect eluate at 1 to 2 mL/minute. (If hexahydrocannabinol as internal standard is being used it will also elute in this fraction)

5. COLUMN RINSE

Remove collection tubes.

Apply 5 mL 50% CH₃OH; draw through under vacuum.

Apply 100 µL ethyl acetate; draw through under vacuum and allow to dry for 2 minutes under vacuum.

NOTE: Do not exceed 2 minute drying time.

6. ELUTE THC METABOLITE (THC-COOH)

Use either above collection tubes (if co-derivitization is to be performed) or clean ones for separate fractions.

Elute with 2 x 2 mL hexane/ethyl acetate (95:5) (v/v) with 1% acetic acid; collect eluate at 1 to 2 mL/minute.

DERIVATIZATION:

1. THC ALONE:

Add 50 µL chloroform and 50 µL TFA; vortex and cap. Heat at 70 °C for 10 minutes. Cool to ambient temperature. Evaporate to dryness. Reconstitute in 30 µL heptane.

2. COMBINED ELUATES OR THC-COOH ALONE:

Add 50 µL acetonitrile and 50 µL BSTFA (with 1% TMCS). Blanket with N₂ and cap. Mix/vortex. React 20 minutes at 70 °C. Remove from heat source to cool.

NOTE: Do not evaporate BSTFA.

Other acceptable derivatization reagents: BSA, MSTFA, MTBSTFA, PFBBR, TFAA, TMPAH, TMSI

section 3 - ANALYSIS

Inject 1 to 3 µL sample into chromatograph.

Monitor the following ions for BSTFA derivitization (Mass Selective Detection):

<u>THC</u>	<u>d₃-THC</u>	<u>Carboxy-Δ⁹-THC</u>	<u>d₃-Carboxy-Δ⁹-THC</u>
303**	306**	371**	374**
315	318	473	476
386	389	488	491

* Suggested internal standards for GC/MS: d₃-THC and d₃-Carboxy-Δ⁹-THC

* Suggested internal standards for other GC: cannabinol, hexahydrocannabinol, oxyphenbutazone, ketoprofen

** Quantitation ion

For method optimization tips, see p. 8

APPENDICES

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY



Appendix A - TREATMENT OF SERUM, PLASMA, OR WHOLE BLOOD SAMPLES

The following methods can be used to prepare serum, plasma, or whole blood samples, and are used to disrupt protein binding to drugs:*

section 1 - pH ADJUSTMENT

- A. Extreme pH values, such as greater than 9, or less than 3. In this case, buffer strengths greater than or equal to 0.1 M should be used.
- B. pH adjustment in stages (i.e. first buffering to pH 5, then to pH 3).

section 2 - PRECIPITATION

Using a polar solvent, such as acetonitrile, methanol, or acetone (generally 2 parts solvent per part of biological fluid), mix/vortex, then centrifuge down the precipitate, and remove the supernatant which contains the drug.

- A. Drawback: the drug may be in part trapped in the precipitate.
- B. The organic solvent should be diluted with aqueous buffer to reduce the solvent strength and also to insure that the compounds of interest are in the proper ionization state.
- C. Acetonitrile is generally considered to be the most effective solvent for disrupting protein binding.
- D. Lower ratios of organic solvent to biological fluid may also be effective (i.e. 10-30% acetonitrile in plasma).

section 3 - ACID TREATMENT

Biological fluids can be treated with formic acid, perchloric acid, or trichloroacetic acid (i.e. 50 μ L of 0.1 M perchloric acid per 500 μ L plasma, or a 1:1 dilution of the biological fluid with 10% trichloroacetic acid). Disruption of protein binding probably occurs through formation of a formate, perchlorate, or trichloroacetate salt of the protein.

section 4 - INORGANIC SALT TREATMENT

Biological fluids can be treated with salts such as ammonium sulfate or zinc sulfate to precipitate proteins.

section 5 - SONICATION

Sonicate the biological fluid for 15 minutes at room temperature, add an appropriate buffer (as described in the "Specimen Preparation" section of the extraction procedure for the particular drug), vortex 30 seconds, centrifuge at 2000 rpm for 15 minutes, and discard pellet.

- * After treating the biological sample, the specimen volume used in the drug extraction procedure should be taken from the treated sample.

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY



Appendix B - DERIVATIZATION INFORMATION

section 1 - PURPOSE OF DERIVATIZATION

Derivatization of drugs prior to GC injection is important for any of three reasons. First, volatility or stability can be improved. Drugs with functional groups such as -COOH, -OH, -NH₂, and -NH tend to form intermolecular hydrogen bonds, decreasing their volatility. Second, derivatization can minimize interactions between the drugs and the column which may interfere with the analysis. Active hydrogens interact destructively with the column's stationary phase, affecting both reproducibility and peak shape. Derivatization can minimize these interactions as well as improve peak resolution as coeluting compounds are separated. Finally, by increasing the bulk of the compound or by introducing atoms or functional groups that interact strongly with the detector, derivatization can also improve detectability.

section 2 - TYPES OF DERIVATIZATION

SILYL

There are three general types of reactions used to derivatize drug samples for GC analysis: silylation, alkylation, and acylation. Silyl derivatives are probably the most widely encountered for GC applications. They are usually formed by replacement of the active hydrogens with -SiR₃ groups. Most trimethylsilyl and *t*-butyldimethylsilyl derivatives have excellent thermal stability and are amenable to a wide range of injection and column conditions. Silylation reagents and the derivatized compounds are hydrolytically unstable, however, and must be protected from moisture.

ALKYL

Alkyl derivatization replaces active hydrogens with aliphatic or aromatic alkyl groups. Probably the largest application of alkylation for analytical derivatization is the conversion of organic acids into esters, especially methyl esters. The advantage of alkylation in this case is the excellent stability afforded by alkyl derivatives. They can be isolated and stored for extended periods if necessary.

ACYL

In acylation, drugs containing active hydrogens (such as in -OH and -NH groups) are converted to ester and amide derivatives. This type of derivative often produces a greater response to the chromatographic detection system than the parent compound in some ECD, TCD, and FID applications. In MS, acyl derivatives tend to direct the fragmentation patterns of compounds, which can provide useful structural information.

section 3 - COMMON DERIVATIZATION REAGENTS

Derivatization reactions should be quantitative, proceed rapidly, and produce products with the desired properties. As a result, many reagents are in common use and the type of derivatization employed will depend on the drug and the method of detection. Typical derivatization reagents and their abbreviations are shown in Table 2. Derivatization reagents suitable for specific drugs are listed in Table 3.

Table 2. Common Derivatization Reagents

Abbreviation	Reagent	Type
BSA	<i>N,O</i> -bis(trimethylsilyl)acetamide	silyl
BSTFA	<i>N,O</i> -bis(trimethylsilyl)trifluoroacetamide	silyl
DMF-DMA	<i>N,N</i> -dimethylformamide dimethylacetal	alkyl
HFBA, HFBA, HFAA	heptafluorobutyric acid anhydride	acyl
MBTFA	<i>N</i> -methy-bis(trifluoroacetamide)	acyl
MSTFA	<i>N</i> -methyl- <i>N</i> -trimethylsilyltrifluoroacetamide	silyl
MTBSTFA	<i>N</i> -methyl- <i>N</i> -(<i>t</i> -butyldimethylsilyl)trifluoroacetamide	silyl
PFPBr	pentafluorobenzyl bromide	alkyl
PFPA, PFPA, PFAA	pentafluoropropionic acid anhydride	acyl
TFAA	trifluoroacetic acid anhydride	acyl
TFAI	trifluoroacetylimidazole	acyl
TMPAH	trimethylanilinium hydroxide	alkyl
TMSC	trimethylsilyl chloride	silyl
TMSI	<i>N</i> -trimethylsilylimidazole	silyl

Table 3. Suitable Derivatization Reagents for Drugs of Abuse

Drug	Derivatization Reagent(s)	Drug	Derivatization Reagent(s)
amphetamine	BSTFA HFBA MSTFA TFAA	THC metabolites	BSA BSTFA MSTFA MTBSTFA PFBBr PFPA/HFIOH PFPA/PFPOH TFAA TMPAH TMSI
methamphetamine	TFAA	LSD	BSA BSTFA MSTFA TFAI
barbiturates	BSTFA TMPAH DMF-DMA PFBBr	opiates	BSTFA MBTFA PFPA TFAA
benzoylecgonine	BSTFA PFPA	PCP	BSTFA HFBA

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY



Appendix C - NON-CHROMATOGRAPHIC DRUG SCREENING (IMMUNOASSAYS)

Immunoassays are based on the principle of competition between labeled and unlabeled antigen (drug) for binding sites on a specific antibody. Antibodies are protein substances with sites on their surfaces to which specific drugs or drug metabolites will bind. In a typical procedure, known amounts of labeled drug are added to a urine sample with known amounts of antibodies. The mixture is then allowed to incubate, during which time the labeled drug and any unlabeled drug originally present in the urine sample compete for binding sites on the antibody. Immunoassays are designed to be specific for a particular drug or drug class, so a series of assays must be performed in an effective screen for the presence of illicit drugs. Also, because of the nature of antibody-antigen binding, all immunoassays suffer from the potential for cross reactivity a lack of specificity.

section 1 - RADIOIMMUNOASSAYS (RIA)

In Radioimmunoassays, the drug is radioactively labeled. After precipitation and centrifugation of the sample, the amount of drug is indicated by the amount of radioactivity found, since this is proportional to the amount of antigen (labeled drug) bound to the antibody. A positive specimen is identified by comparing radioactive counts to those of a positive control prepared in the same manner as that of the unknown urine. The Abuscreen RIA manufactured by Roche Diagnostics is the RIA system most frequently used for drugs of abuse in the US.

Advantages:

very small concentrations of drug detectable (sensitivity ranges on the order of 1-5 ng/mL); small sample volume; minimal sample preparation; automated pipetting and counting equipment allows for large volume, multiple testing.

Disadvantages:

use of radioactive substances; high costs of reagents and instrumentation; long turnaround time (from 1 to 5 hours); significant cross reactions with codeine.

section 2 - ENZYME IMMUNOASSAYS (EIA)

In enzyme immunoassay, the antigen-antibody complexes need not be separated by centrifugation. The labeled antigen in this case is an enzyme that produces a chemical reaction for the detection of drugs. The detection is based on the competition between unlabeled drug/metabolite and labeled drug/metabolite for bonding sites on the antibody. Urine is mixed with a reagent containing glucose-6-phosphate (G-6-P) and antibodies to the drug, as well as a second reagent containing a drug derivative labeled with G-6-P dehydrogenase. The enzyme-labeled drug is incapable of interacting with the G-6-P when bound to an antibody site. If the enzyme-labeled drug does not bind to the antibody, then it is free to react with the substrate. The drug in the urine sample competes for the limited number of antibody binding sites and thereby proportionately increases the total enzyme activity. Enzymatic activity is therefore directly related to the concentration of the drug present in the urine. The EIA most commonly used in the US is the EMIT manufactured by Syva. Two systems are marketed—the EMIT-d.a.u. for use in laboratories with large sample throughput, and the EMIT-st, a portable system which can be used “on-site.”

Advantages:

short analysis time; easily measured nonradioactive endpoint; no necessary separation of bound and free fractions as in RIA.

Disadvantages:

less sensitive than RIA (but still moderate to good); enzyme/substrate interaction is sensitive to temperature variation and ionic adulterants (i.e. salt); significant cross reactions in some assays.

EXTRACTION OF DRUGS OF ABUSE USING BOND ELUT CERTIFY



Appendix D - REFERENCES

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For additional information about drugs of abuse regulation and research visit:

National Institute of Drug Abuse (NIDA)
<http://www.nida.nih.gov/NIDAHome.html>

Substance Abuse and Mental Health Services Administration (SAMHSA)
<http://www.samhsa.gov/>