

A Rapid iMethod™ Test for Drugs of Abuse Screening

iMethod™ Test for Drugs of Abuse Screening Version 2.0 for Cliquid® Software

Liquid Chromatography coupled to Tandem Mass Spectrometry (LC/MS/MS) has quickly become the technique of choice for both screening and confirmation due to elimination of derivatization and simplification of sample preparation, while offering better sensitivity and selectivity, shorter run times, and the ability to analyze wider polarities and molecular weight range of compounds in a single analysis.

The following description outlines the instrument requirements and expected results obtainable from the AB SCIEX iMethod™ Test for the Screening of Drugs of Abuse in plasma, blood or urine when using an AB SCIEX 3200 QTRAP® instrument. The use of this instrument allows the utilization of an information dependent acquisition consisting of an MRM survey scan that triggers product ion dependent scans that can be used for compound confirmation via library searching. This method has also been developed and verified for use with 4000 QTRAP®

LC/MS/MS system. This iMethod™ Test includes both an iMethod™ test for the screening of 700 drugs of abuse in a single run as well as an MRM catalogue containing over 1250 drugs of abuse and related compounds, with up to 6 transitions per compound. The MRM catalogue can be used to create additional custom screening and/or quantification methods. Please note that the AB SCIEX 1250 compound forensic drug library required for confirmation is sold separately.

A number of example sample preparation procedures are provided, based upon the matrix, the degree of clean up required and/or the preferred mode of ionization. These range from a simple extraction and dilution for urine to liquid-liquid for blood and plasma to solid phase extraction for plasma and urine. Internal standards of Diazepam-D₅ and Doxepin-D₃ at known concentration are added during sample preparation to monitor recovery.

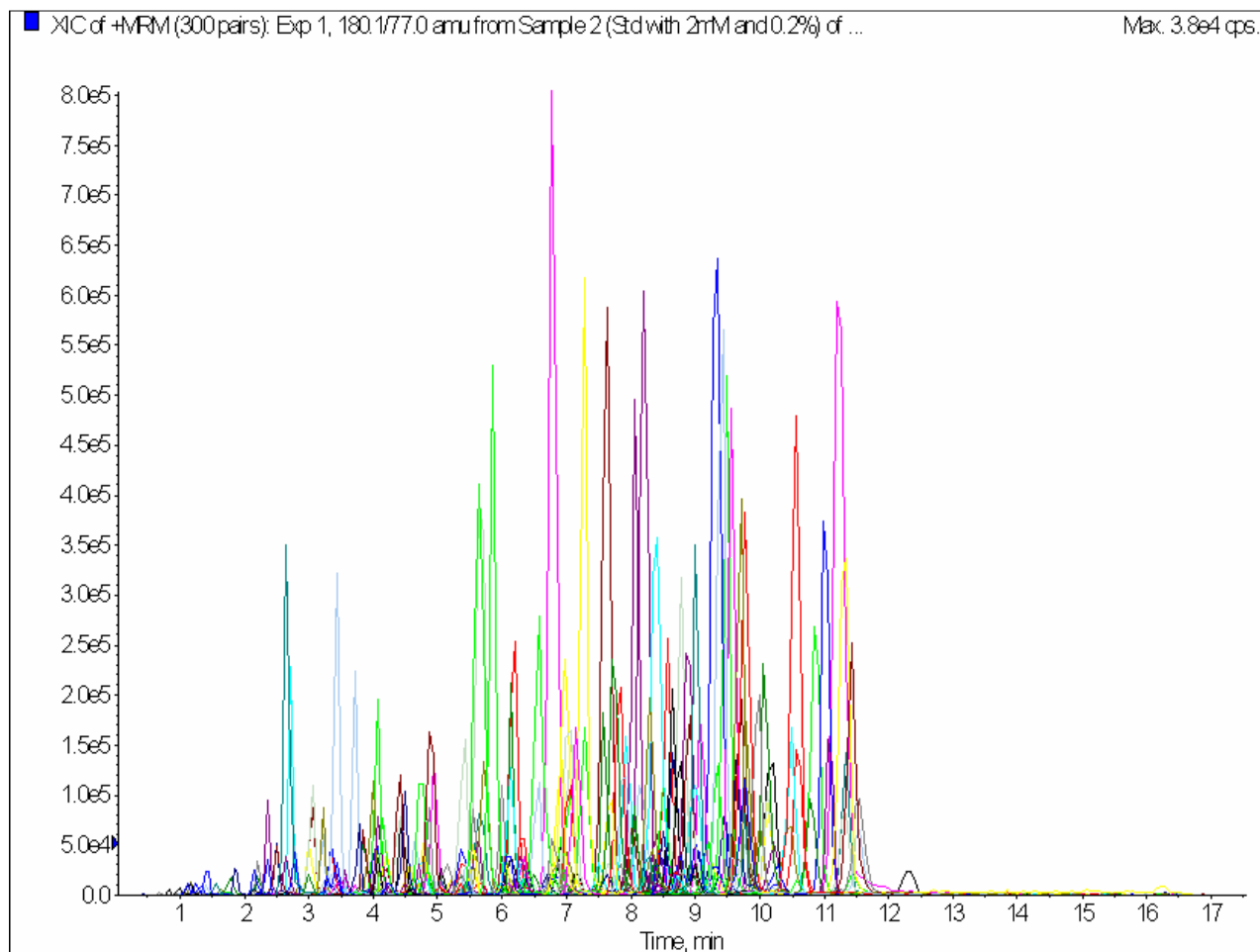


Figure 1: Chromatogram of 300 drugs of abuse and pharmaceuticals detected by LC/MS/MS in Multiple Reaction Monitoring with highest selectivity and sensitivity

Table 1: List of compounds in the 700 compound drug screening method.

Compound name	Compound name	Compound name	Compound name
17-alpha-Methyltestosterone	Amiodarone	Bisoprolol	Cimetidine
2-Amino-5-chlorobenzophenone	Amiphenazole	Bornaprine	Cinnarizine
2-Amino-5-nitrobenzophenone	Amisulpride	Brodifacoum	Cinoxacin
2-Hydroxyethylflurazepam	Amitriptylin	Bromacil	Ciprofloxacin
3,4-Methylenedioxyamphetamine	Amitrole	Bromazepam	Cisapride
3,4-Methylenedioxyethylamphetamine	Amlodipine	Bromocriptine	Citalopram
3,4-Methylenedioxymethamphetamine	Ammoidin	Brompheniramine	Clarithromycin
3,5-Diiodotyrosine	Amorolfine	Brotizolam	Clemastine
4-Benzamidosalicyclic acid	Amoxicillin	Bucetin	Clenbuterol
6-Mercaptourine	Amphetamine	Bufexamac	Clobazam
6-O-Monoacetylmorphine	Amrinone	Bumetanide	Clobenzepam
7-Aminoclonazepam	Antazoline	Bunazosin	Clobutinol
7-Aminodesmethylflunitrazepam	Apalcillin	Bunitrolol	Clomethiazole
7-Aminoflunitrazepam	Apomorphine	Bupivacaine	Clomipramine
7-Aminonitrazepam	Apophedrin	Bupranolol	Clonazepam
9-Hydroxyrisperidone	Apraclonidin	Buprenorphine	Clonidine
Acebutolol	Aprinidine	Buprofezin	Clopenthixol
Aceclidine	Atenolol	Buspirone	Clopidogrel
Aceclofenac	Atorvastatin	Butaperazine	Cloprednol
Acemetacin	Atraton	Butetamate	Clozapine
Acepromazine	Atrazine-desethyl	Butoxycaine	Cocaine
Aceprometazine	Atropine	Cafaminol	Codeine
Acetiamin	Atropinmethylbromid	Caffeine	Coniine
Acetylamino nitroprophoxybenzene	Azatadine	Candesartan	Corticosterone
Aciclovir	Azelastine	Caproylresorcinol	Cortisone
Actinoquinol	Azintamid	Captopril	Cotinin
Adenine	Aztreonam	Carazolol	Coumatetrayl
Adrenalone	Baclofen	Carbamazepine	Croconazole
Ajmaline	Bambuterol	Carbamazepine 10,11-epoxide	Cromoglicicacid
Alachlor	Bamifylline	Carbendazim	Cyamemazine
Alclometasone-17,21-dipropionate	Bamipin	Carbinoxamine	Cyclicine
Alimemazine	Beclamide	Carbutamide	Cyclophosphamide
Alizapride	Beclometasone dipropionate	Carbuterol	Cyclovalone
Allopurinol	Befunolol	Carteolol	Cyproheptadine
Almitrine	Bendiocarb	Carvedilol	Cytarabine
alpha-Hydroxyalprazolam	Benfluorex	Celiprolol	D3-Doxepin
alpha-Hydroxytriazolam	Benodanil	Cerivastatin	D5-Diazepam
Alprazolam	Benperidole	Cetirizine	Dapiprazole
Alprenolol	Benproperine	Chinine	Deflazacort
Altretamine	Bensultap	Chlorazaniil	Demeton-S-methylsulfone
Alypin	Bentiromide	Chlorbenzoxamine	Denaverine
Amantadine	Benzatropine	Chlorcyclizine	Desalkylflurazepam
Ambroxol	Benzocaine	Chlordiazepoxide	Desipramine
Ambucetamide	Benzocetamine	Chloridazon	Desmedipham
Amezinium	Benzododecinium	ChlormadinoneAcetate	Desmethylclobazam
Amidopyrin	Benzoyllecgonine	Chlormequat	Desmethylclomipramine
Amidotrizoic Acid	Benzthiazuron	Chlorphenethiazine	Dexamethasone
Amiloride	Berberine	Chlorpheniramine	Dexamethasone 21-isonicotinate
Aminodantrolene	Betamethasone 21-phosphate	Chlorpromazine	Dexfenfluramine
Aminoglutethimide	Betaxolol	Chlorpromazine Sulfoxide	Dextromethorphan
Aminophenazone	Bevonium	Chlorprothixene	Dextropropoxyphene
Aminopromazine	Bezafibrate	Cilastatin	Diaveridine
Aminorex	Biperiden	Cilazapril	Diazepam

Compound name	Compound name	Compound name	Compound name
Dibutyladipate	Fenpipramide	Hydrocortisone	Mefenorex
Diclofenac	Fenpiprane	Hydrocortisone 21-acetate	Mefexamide
Dicycloverine	Fenproporex	Hydromorphone	Mefloquine
Dienogest	Fentanyl	Hydroxyzine	Melatonin
Diethazine	Fenticonazole	Hymecromone	Melitracen
Diethylcarbamazine	Fexofenadine	Imipramine	Meloxicam
Difenoconazole	Flecainide	Indanazoline	Melperone
Difenoxuron	Flocoumafene	Indapamine	Melphalan
Diflucortolone	Floctafenine	Indinavir	Mepindolol
Dihydrocodeine	Fluanisone	Indoprofen	Mepivacaine
Dihydroergocristin	Fluconazole	Indoramin	Meptazinol
Dihydroergotamine	Flumazenil	Iopodicacid	Mequitazine
Dilazep	Flunarizine	Ipratropium	Mescaline
Diltiazem	Flunitrazepam	Iprazochrome	Mesoridazine
Dimetindene	Fluoxetine	Irbesartan	Mesuximide
Dimetotiazine	Flupentixol	Isoaminile	Metaclazepam
Dimetridazole	Fluphenazine	Isoconazole	Metamfepramone
Dioxethedrin	Flupirtine	Isoprenaline	Metamitron
Diphenamid	Flurazepam	Isoproturon	Metamphetamine
Diphenhydramine	Flurochloridone	Isothipendyl	Metazachlor
Diponium	Fluspirilen	Isoxicam	Metenolone acetate
Diprophylline	Fluticasone Propionate	Isoxsuprine	Metformin
Dipyridamole	Fluvastadine	Kavain	Methabenzthiazuron
Disopyramide	Fluvoxamine	Ketamine	Methadone
Dixyrazine	Fuberidazole	Ketoprofen	Methaphenilene
Dobutamine	Furalaxyl	Ketorolac	Methapyrilene
Dorzolamide	Gabapentin	Ketotifen	Methaqualon
Doxapram	Galantamine	Lamotrigine	Methazolamide
Doxepin	Gallopamil	Lercanidipine	Methfuroxam
Doxylamine	Gemcitabine	Levocabastine	Methocarbamol
Drofenine	Glibenclamide	Levodopa	Methoprotryne
Ecgoninemethylester	Glibornuride	Levomepromazine	Methotrexate
EDDP	Gliclazide	Levopropylhexedrin	Methylephedrine
Embutramide	Glimepiride	Lidocaine	Methylphenidate
Enalapril	Glipizide	Lisinopril	Methylscopolamine
Enoximone	Gliquidone	Lonazolac	Methylthiouracil
Ephedrine	Griseofulvin	Loperamide	Metipranolol
Eprosartan	Guaifenesin	Loratadine	Metixene
Esculin	Guanabenz	Lorazepam	Metoprolol
Esmolol	Guanethidine	Lormetazepam	Metronidazole
Estazolam	Guanfacine	Losartan	Metsulfuron-methyl
Ethenzamide	Guanoxan	Loxapine	Metyrapone
Etofenamate	Haloperidol	Lysergide	Mexiletine
Etomidate	Haloxypop ethoxyethyl ester	Mafenide	Mianserin
Etoposide	Heptaminol	Maprotiline	Miconazole
Famotidine	Heroin	MBDB	Midazolam
Fedrilate	Hexazinone	Mebeverine	Midodrine
Felbamate	Hexobendine	Meclizine	Milrinone
Felodipine	Histamine	Meclofenamic acid	Minoxidil
Fenarimol	Histapyrrodine	Mecloxamine	Mirtazapine
Fendiline	Homatropine	Meclozine	Mizolastine
Fenetylline	Hordenine	Medazepam	Moclobemide
Fenfluramine	Hydrocodone	Mefenamic acid	Modafinil
Molsidomine	Ondansetron	Pirbuterol	Ritodrine

Compound name	Compound name	Compound name	Compound name
Monocrotophos	Pipramol	Pirenzepine	Rizatriptan
Monolinuron	Orciprenaline	Piretanide	Ropinirole
Moperone	Ornidazole	Piritramide	Ropivacaine
Morphine	Orphenadrine	Piroxicam	Rosiglitazon
Morphine-3-β-D-glucuronide	Oxadixyl	Pizotifen	Salbutamol
Moxaverine	Oxamyl	Practolol	Salicylamide
Moxisylyte	Oxatomide	Prajmalium	Scopolamine
Moxonidine	Oxazepam	Pramipexole	Serotonin
Nabumetone	Oxcarbazepine	Prazepam	Sertindole
Nadolol	Oxeladin	Prazosin	Sertraline
Naftidrofuryl	Oxetacaine	Prednisolone	Sildenafil
Naftifine	Oxiconazole	Prednisone	Simazine
Nalidixic acid	Oxilofrine	Prilocaine	Sotalol
Nalorphine	Oxitriptan	Primaquine	Spirapril
Naloxone	Oxitropium	Primidone	Stanozolol
Naltrexone	Oxomemazine	Procainamide	Sulfabenzamide
Nandrolone	Oxprenolol	Prochlorperazine	Sulfadiazine
Naphazoline	Oxybuprocaine	Procyclidine	Sulfadoxine
N-Despropylpropafenone	Oxybutynin	Progesterone	Sulfaethidole
Nebivolol	Oxycodone	Promazine	Sulfaguanidine
Nefopam	Oxyfedrine	Promethazin	Sulfamethizole
Nicardipine	Oxymetazoline	Prometryn	Sulfamethoxazole
Nicotinamide	Oxymorphon	Propafenone	Sulfapyridine
Nicotine	Oxypertine	Propiconazole	Sulfaquinoxaline
Nifedipine	Papaverine	Propionylpromazine	Sulindac
Nifenazone	Paracetamol	Propipocaine	Sulpiride
Niflumic acid	Paraoxon	Propiverine	Sultiame
Nimodipine	Paroxetine	Propranolol	Sumatriptan
Nimorazole	Penfluridol	Propyphenazone	Suxibuzone
Nisoldipine	Pentamidine	Prothiopendyl	Tadalafil
N-Isopropylsalicylamide	Pentoxyverine	Protriptyline	Talinolol
Nitrazepam	Perazine	Pseudoephedrine	Tamoxifen
Nitrendipine	Periciazine	Psilocin	Telmisartan
Nizatidine	Perindopril	Pyranocoumarin	Temazepam
N-Methylephedrine	Perphenazine	Pyribenzamine	Tenoxicam
Nomifensine	Pethidine	Pyridoxine	Terazosine
Norbuprenorphine	Phenacetin	Pyrimethamine	Terbinafine
Nordiazepam	Phenazone	Pyritinol	Terbutaline
Nordiazepam N-ethyl	Phenazopyridine	Pyrvinium	Terbutryn
Norephedrine	Phencyclidine	Quetiapine	Terconazole
Norethisterone	Phenelzine	Quinapril	Terfenadine
Norethisterone acetate	Pheniramine	Quinine	Tertalolol
Norfefrine	Phenprocoumon	Ramipril	Tetracaine
Norfentanyl	Phenylephrine	Ranitidine	Tetrazepam
Norfloxacin	Phenyltoloxamine	Raubasine	Tetroxoprim
Normorphine	Phenytoin	Reboxetine	Tetryzoline
Nortriptyline	Pholedrine	Remoxipride	THC
Noscapine	Physostigmine	Repaglinide	THC-COOH
Nuarimol	Pilocarpine	Reproterol	THC-OH
Obidoxime	Pindolol	Reserpine	Thebacon
Ofloxacin	Pioglitazone	Riluzole	Theobromine
Olanzapine	Pipamperone	Risperidone	Theophylline
Thiabendazole	Tocainide	Triazolam	Urapidil
Thiamazole	Tolazoline	Trifluoperazine	Valdecoxib

Compound name	Compound name	Compound name	Compound name
Thiamine	Tolbutamide	Trifluoperidol	Valsartan
Thiopropazate	Toliprolol	Triflupromazine	Vardenafil
Thiopropazine	Tolnaftate	Trimethobenzamide	Venlafaxine
Thioridazine	Tolpropamine	Trimethoprim	Verapamil
Thiram	Tolycaine	Trimipramine	Vincamine
Tiagabine	Topotecan	Tripelennamine	Warfarin
Tiapride	Torasemide	Tripolidine	Xantinol
Ticlopidine	Tramadol	Tritoqualine	Xipamide
Tiemonium	Tranexamic acid	Tromantadine	Xylometazoline
Tilidine	Trazodone	Tropisetron	Yohimbine
Timolol	Triadimenol	Trospium	Zolpidem
Tinidazole	Triallate	Tryptamine	Zopiclone
Tizanidine	Triamterene	Tulobuterol	Zuclophenthixol

Results

An example chromatogram for drugs of abuse and screening is shown in Figure 1 to highlight the possibility of detecting hundreds of compounds in a single experiment. An example reports generate automatically after analysis by Cliiquid® Software is presented in Figure 2.

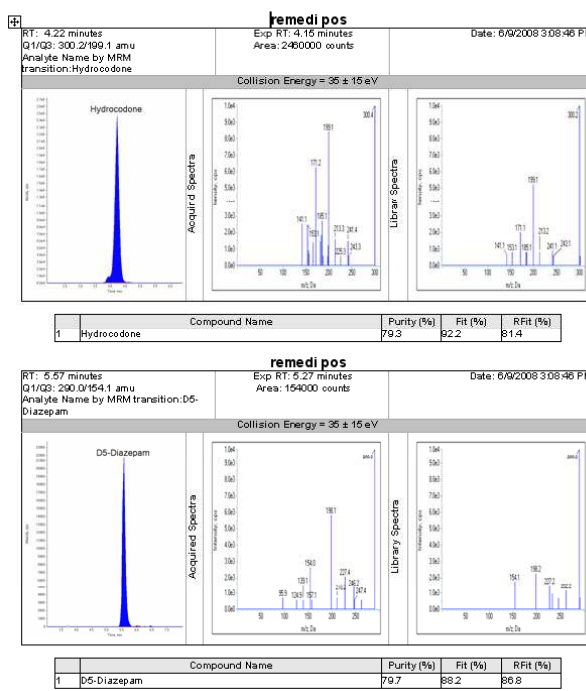


Figure 2: Example screening report.

System Requirements

In order to run this method as outlined above, the following equipment and reagents are required:

- An AB SCIEX 3200 QTRAP® or 4000 QTRAP® LC/MS/MS System
- A Shimadzu Prominence 20A LC System with reservoir tray and bottles, system controller CBM-20A, 100 µL mixer, 2 isocratic pumps LC-20AD, 3 channel degasser Autosampler SIL-20AC, column oven CTO-20AC or Agilent 1100/1200 LC system with binary pump G1312A (without static mixer), well plate auto sampler, and thermostated column oven.
- LC/MS grade water, acetonitrile, ammonium formate and formic acid
- 1.5 mL Eppendorf tubes
- A Restek pre-column, 5µm 60Å, PFP Propyl, 10 x 2.1 mm
- A Restek analytical column, 5µm 60Å, PFP Propyl Column, 50 x 2.1 mm (included in the iMethod Test)
- SPE cartridges, 200mg Chromabond® Macherey-Nagel
- A centrifuge able to accommodate Eppendorf tubes and run at 14000 rpm
- Pipettes and standard laboratory glassware

Ordering Information

Product Name	Part Number
iMethod™ Test for Drugs of Abuse Screening Version 2.0 for Cliiquid® Software	1040049

While the information provided above outlines the instrument requirements and expected results obtainable from the AB SCIEX iMethod™ Test for the Analysis of Drugs of Abuse, please note that the results obtained do require some experience with LC/MS/MS and sample preparation procedures. As such, web-based and on-site training are available to assist in the deployment of the iMethod™ Test and are recommended for inexperienced users. Please consult your local sales representative for more details.

Important Note

The iMethod™ Test described above has been designed by AB SCIEX to provide the sample prep and instrument parameters required to accelerate the adoption of this method for routine testing. This method is provided for information purposes only. The performance of this method is not guaranteed due to many different potential variations, including instrument performance, tuning, and maintenance, chemical variability and procedures used, technical experience, sample matrices, and environmental conditions. It is up to the end user to make adjustments to this method to account for slight differences in equipment and/or materials from lab to lab as well as to determine and validate the performance of this method for a given instrument and sample type. Please note that a working knowledge of Analyst® Software may be required to do so.

The purchase and use of certain of the chemicals listed below may require the end user to possess any necessary licenses, permits or approvals, if such are required in accordance with local laws and regulations. It is the responsibility of the end user to purchase these chemicals from a licensed supplier, if required in accordance with local laws and regulations. The suppliers and part numbers listed below are for illustrative purposes only and may or may not meet the aforementioned local requirements. AB SCIEX is not responsible for user's compliance with any statute or regulation, or for any permit or approval required for user to implement any iMethod™ procedure.

The information included in this product is intended for reference and research purposes only. AB SCIEX offers no guarantee as to the quality or suitability of this data and suitability of the information included in this (Library, database, etc.) for use with your specific application.

For Research Use Only. Not for use in diagnostic procedures.

© 2010 AB SCIEX. The trademarks mentioned herein are the property of AB Sciex Pte. Ltd. or their respective owners. AB SCIEX™ is being used under license.

Publication number: 1710210-01