A Rapid Method for Detection of Drugs of Abuse in Blood Samples Using the Thermal Separation Probe and the 5975T LTM GC/MS

Application Note

Forensics

Authors

Suli Zhao
Agilent Technologies, Inc.
Shanghai
China
Runsheng Zhang
Institute of Forensic Science
Municipal Public Security Bureau,
Shanghai
China

Abstract

A new and rapid qualitative method for detection of drugs in whole blood was developed using the innovative thermal separation probe (TSP) sample introduction technique on the transportable Agilent 5975T LTM GC/MS. No sample cleanup was required, and Deconvolution Reporting Software (DRS) software streamlined data handling. Of the 56 thermally stable drugs analyzed, >94% had a method detection limit of 5 ng/µL.

Introduction

Clinical and forensic questions regarding drugs of abuse can be solved more effectively when, in addition to the analytical results of urine samples, a quantitative determination can be made in blood. However, forensic toxicologists are routinely confronted with the difficult problem of detecting and quantitating drugs of abuse in postmortem blood due to the complexity of the blood matrix and the wide range of drugs that must be tested for. The list of target compounds to be screened can number in the hundreds. Fortunately, most drugs and their metabolites are among the structures which can be analyzed by GC/MS, and identification of drugs of abuse by GC/MS spectral data is accepted as evidence in courts of law.

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This application note describes a rapid and low-cost method for the qualitative detection of a total of 63 drugs of abuse in whole blood using the TSP with the Agilent 5975T LTM GC/MS. No sample preparation is required, and the non-volatile components of the blood remain inside the TSP micro-vial after the drug compounds are volatilized. Not only does a simple extraction procedure lower cost by saving time, it also reduces the chances of human error. The Low Thermal Mass (LTM) technology on the GC also reduces the run time by heating and cooling the column very efficiently for significantly shorter analytical cycle times as compared to conventional air-bath GC ovens.

Experimental

Reagents and Standards

All reagents were analytical or HPLC grade. The drug standards were provided by Shanghai Public Security Bureau, China. These standards were spiked into whole bold at concentrations varying from 0.1 to 40 ng/µL to test the sensitivity of the method for each drug.

Instruments

This method was developed on the Agilent 5975T LTM GC/MS system using a split/splitless inlet and the TSP. The instrument conditions used are shown in Table 1.

Data Analysis

Agilent Deconvolution Reporting Software (DRS) for GC/MS was used for compound identification, combining results from the Agilent MSD Productivity ChemStation, the NIST Automated Mass Spectral Deconvolution and Identification Software (AMDIS), and the NIST 2008 Mass Spectral Search Program (NIST08).

Samples

The blood samples were obtained from the Shanghai Public Security Bureau.

Sample Preparation

For the spiked blood samples as well as samples from drug abusers in which the drug concentrations were expected to be very high, the TSP micro-vial was filled to less than half its total volume with dehydrated Na_2SO_4, and 3–5 µL of whole blood was spotted directly onto the Na_2SO_4.

Table 1. GC/MS Run Conditions

GC run conditions	
Guard column	1 m column with same phase as analytical column, connected to the injector
Analytical column	Agilent HP-5ms LTM 10 m × 0.18 mm, 0.18 µm, ordered as custom p/n 100–2000LTM
Injection volume	1 µL
Inlet temperature	Isothermal at 280 °C
Injection mode	Split, 10:1
LTM temperature gradient	12 second hold at 90 °C 90 °C to 320 °C at 60 °C/min Hold at 320 °C for 2.75 min
Isothermal temperature	280 °C
Carrier gas	Helium
Transfer line temp	280 °C
MS conditions	
Ion source temperature	230 °C
Quadrupole temperature	150 °C
Ionization	EI mode
Scan mode	Full scan, *m/z* 30–500
EMV mode	Gain factor
Gain factor	5.00
Resulting EM voltage	1129 V
Solvent delay	0.5 min

Results and Discussion

Fast Method Selection

The Agilent GC MXLATOR method translation software tool was used to find operating conditions to accelerate a method that was obtained from a customer using the Agilent Deconvolution Reporting Software (DRS) Solution for Forensic Technology (refer to document G1674-90000) and an Agilent HP-5ms LTM 10 m × 0.18 mm, 0.18 µm column. The translated method reduced the running time to 6.5 minutes, versus a run time of more than 28 minutes for the original method without significantly sacrificing the resolution, compared to the method using a 30 meter standard column. Good peak shape was obtained for the drugs listed in Table 2, without peak discrimination (Figure1). Using the LTM column module was a key to enabling the short separation time. Rapid temperature programming rates of up to 1200 °C/min can be set, and retention time repeatability is comparable to conventional GC.

Table 2. Drugs Analyzed Using the Fast Method in Figure 1

Retention Time (min)	Drug
1.8765	Barbital
2.3049	Amobarbital
2.4384	Secobarbital
3.1265	Cocaine
3.2050	1-Piperidinepropanol, alpha.-cyclopentyl-.alpha.-phenyl-
3.2407	Promethazine
3.2788	SKF525
3.3232	Oxazepam
3.4365	Lorazepam
3.4782	Diazepam
3.563	Chlorpromazine
3.5645	Chlorprothixene
3.5950	Chlordiazepoxide
3.9862	Papaverine
4.024	Clozapine
4.0243	Clonazepam
4.0971	Estazolam

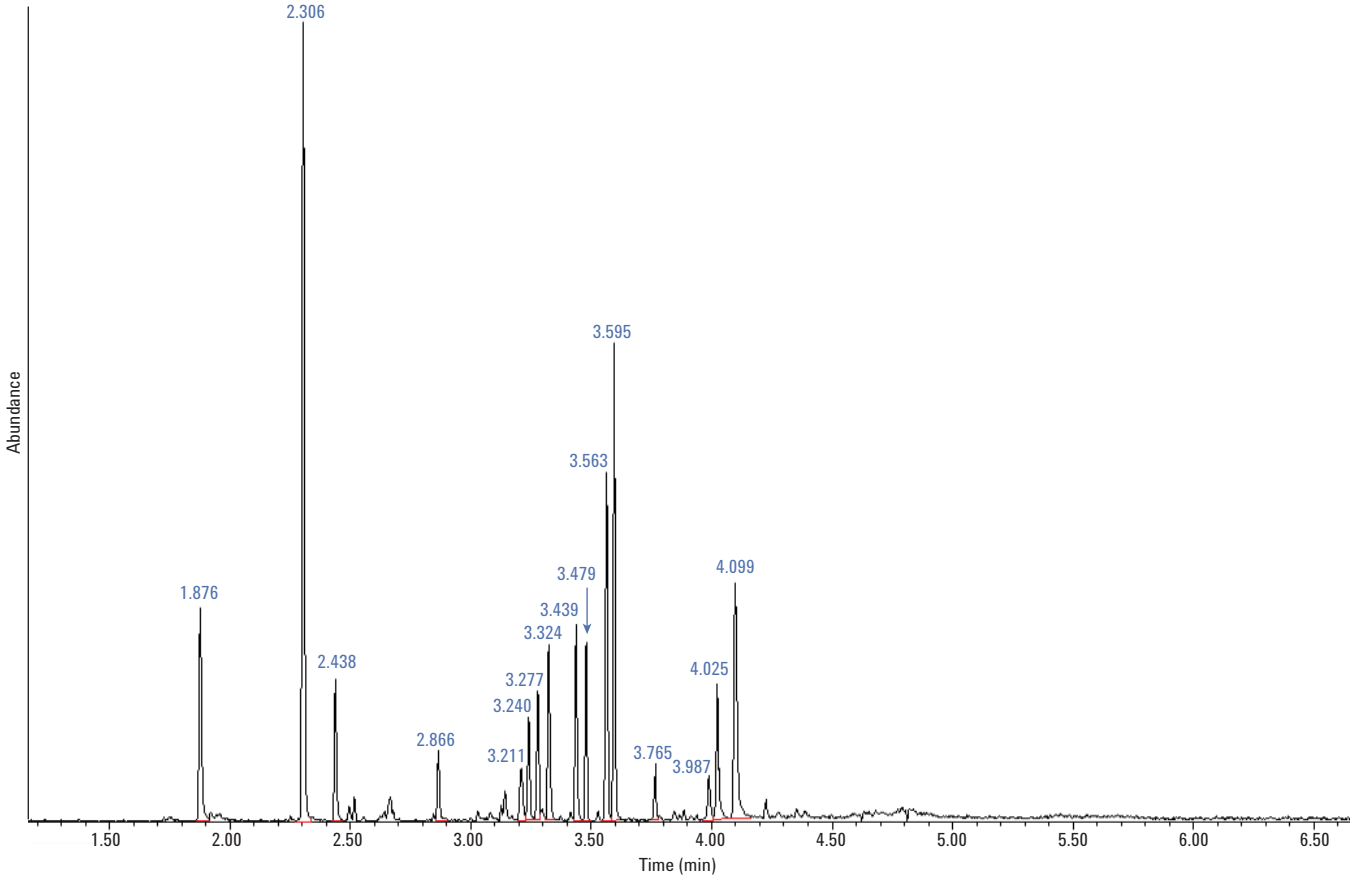

Figure 1. Total ion current (TIC) of drug standards spiked into solvent using the new fast method with TSP.

Drug Detection in Whole Blood

A 3 µL sample from a fatal overdose subject was used to test the method (Figure 2). The baseline is low and very smooth late in the temperature program, implying that the volatile components in blood can be eluted completely with this method. The tallest peak in this sample is cholesterol, and the other large, visible peaks are primarily long-chain fatty acids. The drugs of abuse are hidden in these large peaks. With the help of DRS and the NIST AMDIS, the drugs of abuse present in this sample were identified accurately. Figure 3 shows the deconvolution result for the drug chlorpromazine.

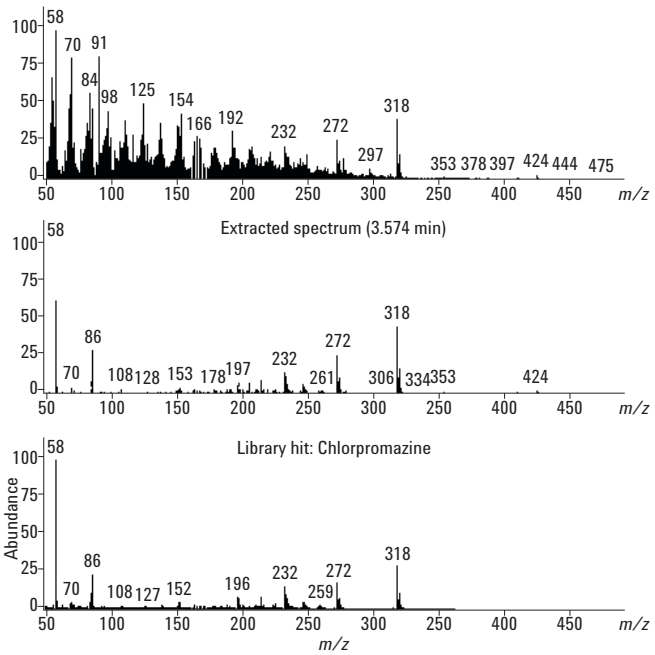

Figure 3. Deconvolution of the peak at 3.574 minutes and its subsequent identification as chlorpromazine. The top spectrum is the sample spectrum at retention time 3.574 minutes; the middle spectrum is the sample spectrum after deconvolution with DRS, and the bottom spectrum is the NIST library spectrum for Chlorpromazine, providing a positive identification of Chlorpromazine.

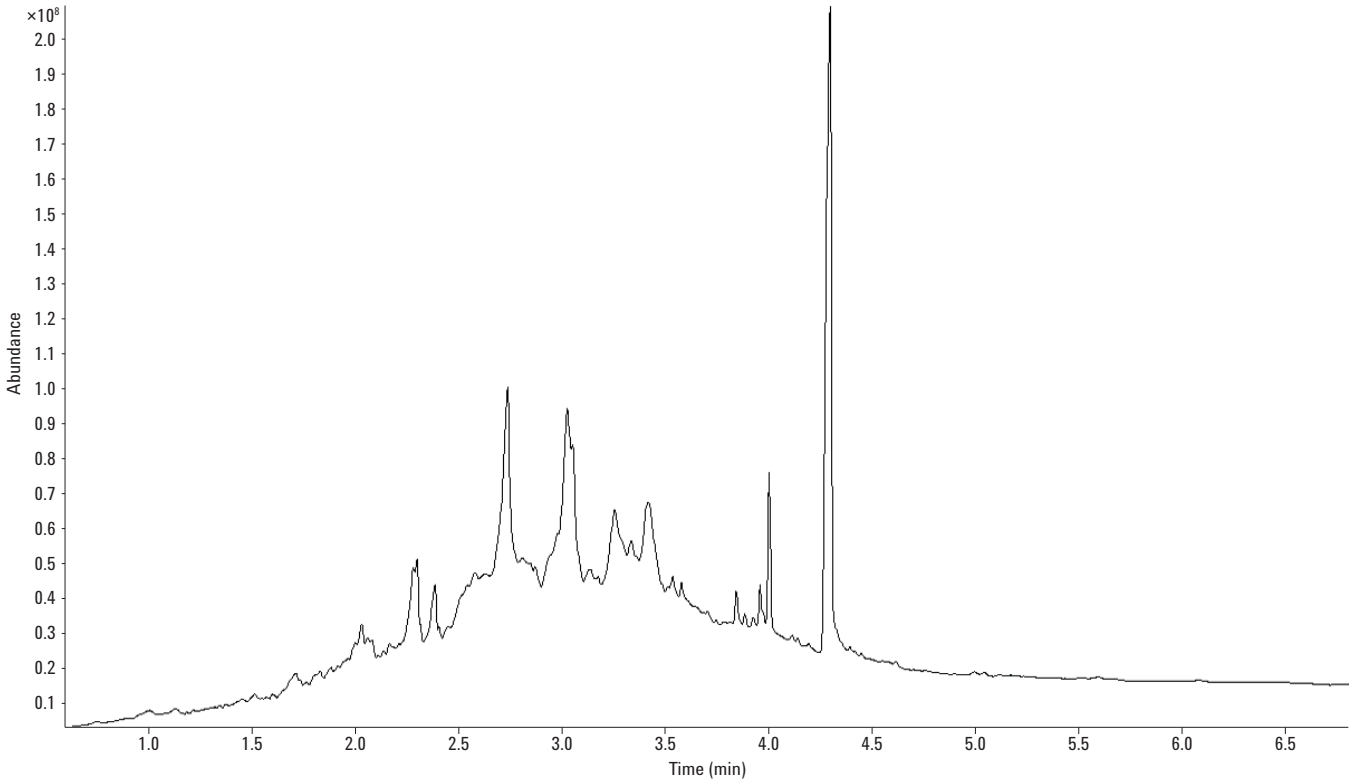

Figure 2. TIC chromatogram from a blood sample taken from an overdose subject.

Method Confirmation

We selected 63 drugs (Table 3) as the targets to explore the detection limits of the method. Each drug was tested at 0.1, 0.5, 1.0, 5.0, 20.0, and 40.0 ng/µL spiked into whole blood. The DRS library was used to confirm the targets. The results showed that some drugs are easily decomposed, including Iso-LSD, Melatonin, 2 C-B, Aminorex and Perphenazine (Figure 4). The target peak is very weak for each of these drugs and has a distinctive tail. There are also many small peaks, indicating decomposition. For these compounds, high concentrations (≥ 20 ng/µL) were required to confirm the targets. Two compounds (Pemoline and benzoylecgnine) decomposed significantly, and no target peak was observed for either drug. However, more than 80% of the compounds could be confirmed by this method at 5 ng/µL, and this concentration was set as the method detection limit (MDL).

Table 3. Method Detection Limits for 63 Drugs Identified in Whole Blood with the Fast Method

Name	0.1 ng/µL	0.5 ng/µL	1 ng/µL	5 ng/µL	20 ng/µL	40 ng/µL
Codeine						
Morphine						
Lidocaine						
Amitriptyline						
Imipramine						
Chlorpromazine						
Clozapine						
Estazolam						
Doxepin						
Aprobarbital						
Trifluoperazine						
Midazolam						
Chlordiazepoxide						
Fenfluramine						
Phenylpropanolamine						
Ibuprofen						
Dextropropxyphene napsylate						
Iso-LSD						
Pemoline						
Propafenone						
Amphetamine sulfate						
Ecgonine methyl ester						
3,4-Methylenedioxy-amphetamine						
Katamine						
Buprenorphine						
Methadone						
Fentanyl						
Caffeine						
Tramadol						
Flunitrazepam						
Flurazepam						
Carbamazepine						
Dihydrocodeine						
Aminorex						
Norcocaine						
Prazepam						
Pheniramine						

Green shading indicates that the drug could be positively identified at the denoted concentration.

Table 3. Method Detection Limits for 63 Drugs Identified in Whole Blood with the Fast Method (continued)

Name	0.1 ng/µL	0.5 ng/µL	1 ng/µL	5 ng/µL	20 ng/µL	40 ng/µL
Lofexidine hydrochloride						
Diphenylhydramine						
Melatonin						
Mescaline						
Benzoylecgnine						
Ecgonine ethyl ester						
Norketamine						
Hydrocodone						
Lorazepam						
2C-B						
PCP						
Phenobarbital						
Amobarbital						
Barbital						
Secobarbital						
Alprazolam						
Methamphetamine						
Perphenazine						
Nitrazepam						
Lorazepam						
Noscapine						
Diazepam						
Oxazepam						
Estazolam						
MDMA						
Triazolam						

Green shading indicates that the drug could be positively identified at the denoted concentration.

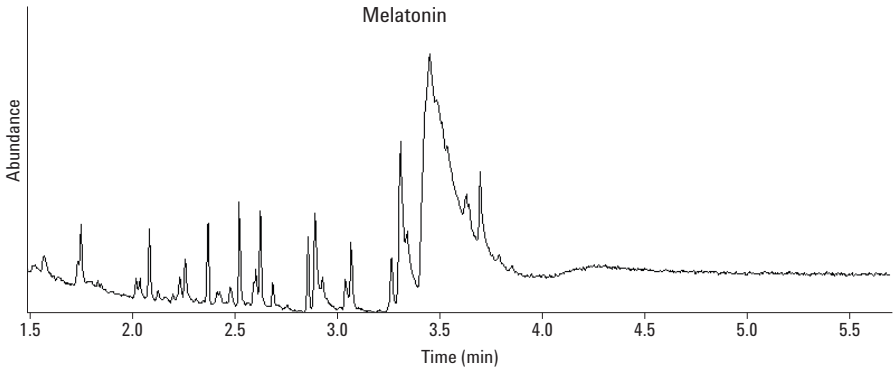

Figure 4. The TIC chromatogram for melatonin at 1,000 µg/mL, illustrating significant decomposition of the drug.

Conclusion

An innovative and rapid qualitative method for detecting drugs in whole blood has been developed using the LTM technology on the portable Agilent 5975T LTM GC/MS and the TSP sample introduction technique. The method eliminates time-consuming and expensive manual sample cleanup that could be difficult to perform at a remote site and introduce error. DRS software obviated the need for sample preparation by providing deconvolution and identification in the complex whole blood matrix. Of the 63 drugs analyzed, 56 were not thermally labile, and over 94% of these had a method detection limit of 5 ng/µL.

For More Information

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