Automatic Screening of Major Impurities in Methamphetamine Samples Synthesized by Emde Method Using Accurate Mass Spectrometry

Zhendong Hua[1], Wei Jia[1], Fengyun Pan[2], Hua-fen Liu[2];
[1] Drug Intelligence and Forensic Center of Minister of Public Security, Beijing,China; [2] Applications Support Center, ABSciex, Beijing, China;

ABSTRACT

An ultra-fast liquid chromatography coupled with hybrid quadrupole time-of-flight tandem mass spectrometry method which featured high resolution, mass accuracy and sensitivity, was developed to detect impurities for profiling in methamphetamine samples synthesized by Emde method. In this study, electrospray ionization in positive ion mode with full scan of TOF MS-IDA-MSMS was used to determine impurities. After analysis of two hundred samples, sixteen impurities as markers were selected by MarkerView™ software based on the criterion of (1) present in at least 10% samples, (2) with at least one nitrogen, (3) with degree of unsaturation over 4. Major impurities' structure were elucidated based on TOF MS and MS/MS spectra. Case to case comparison method was also successfully developed by Pearson correlation analysis of their relative contents in different seizures.

INTRODUCTION

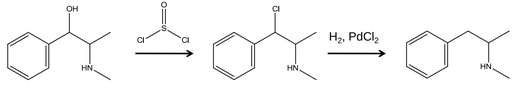

Fig.1 Methamphetamine synthesized by Emde method

Methamphetamine is one of the most widely abused drugs in China. Ephedrine and pseudoephedrine are main precursors for methamphetamine manufacture in clandestine labs. Two synthetic routes, i.e. Nagai method with iodine & red phosphorus and Emde method (Fig.1) with thionylchloride & hydrogen gas, are frequently used. The Nagai method resulted in methamphetamine with abundant impurities, which could be easily profiled by GC-MS with the method developed by "CHAMP" program [1]. However, methamphetamine synthesized through Emde method is usually of high purity, thus few impurities could be detected by GC-MS. Therefore, a profiling method for methamphetamine using Emde method is essential to support law enforcement agency for both evidential and strategic intelligence purposes.

In this study, an ultra-fast liquid chromatography coupled with hybrid quadrupole time-of-flight tandem mass spectrometry method which featured high resolution, mass accuracy and sensitivity was developed to screen major impurities in methamphetamine. 200 samples, whose synthetic routes were confirmed to be emde method by ICP-MS, were analyzed by of TOF MS-IDA-MSMS. Then the data collected were processed with MarkerView software, and sixteen impurities as markers were found to frequently present in these samples. Meanwhile, the contents of these markers varied notably in different samples, which facilitated the subsequent development of correlation analysis for sample to sample comparison. The chemical formulas of these markers were determined by the accurate mass measured, and some of their structures were deduced by the fragment mass spectrum. Next preparative, we plan to collect or synthesis some of the impurities for further verification.

MATERIALS AND METHODS

Sample Preparation:

Sample preparation method was from "Collaborative Harmonization of Methods for Profiling of Amphetamine Type Stimulants" (CHAMP) [1] with minor modifications. We focused on basic impurities in the sample.
Organic Impurity was prepared by liquid-liquid extraction.

Sample Buffer was 1M Tris base Buffer with pH 8.1
Dissolve 200 mg methamphetamine in 4 mL Tris buffer
Centrifuge the tubes for 5 min at 2000 rpm to separate the phases
Take an aliquot of the toluene layer and place it in a LC sample vial. Injection volume is 0.2uL
.

LC/MS Experiment and Workflow
AB SCIEX TripleTOF® 5600 with Shimadzu Nexera LC-30A® System.
An Agilent Eclipse Plus C18 (100 x 2.1mm, 1.8μm) Column was used at 40° C. Mobile phases were acetonitrile (B) and water/acetonitrile (95/5) +0.1% formic acid+2mM ammonium formate (A) at a flow rate of 0.4mL/min.
Valve was switched to waste from 2.6-3.4min (large peak of methamphetamine)
LC time program

Time(min)		Parameter
0.5	Bconc	5
12.5	Bconc	100
15	Bconc	100
15.5	Bconc	5
20	Bconc	5

MS Instrument Parameters:

Ion Source: ESI		Polarity:	Positive
TOF MS-IDA-10MS/MS		Curtain Gas	30
TOF MS m/z	100-1000	GS1	50
MS/MS m/z	100-1000	GS2	50
Tempeture	600	DP	80
CE	35 (MS2)	CES	15

PeakView® software was used to perform accurate mass data processing and MarkerView software for statistics analysis to seek markersh

Statistics criterion
(1) present in at least 10% samples,
(2) with at least one nitrogen,
(3) with degree of unsaturation over 4.

RESULTS

A total of 16 markers were found and their MS and MS/MS spectra were acquired with high quality using the TOF MS IDA-MSMS scan.

Markers	Formula	Theoretical Mass $[M+H]^+$	TripleTOF mass error (ppm)	Retention time(min)
1	C12H19N	178.1590	0.5	3.68
2	C11H17NO	180.1383	0.2	3.7
3	C12H18N2	191.1543	-0.6	3.75
4	C11H17N	164.1434	-1.2	3.77
5	C10H21N	156.1747	0.8	4.5
6	C29H38N2	415.3108	-1.8	4.82
7	C20H28N2O	313.2274	0.1	5.19
8	C20H28N2O	313.2274	-1.2	5.69
9	C11H13NO2	192.1019	-0.1	5.91
10	C21H28N2O	325.2274	-0.9	5.93
11	C12H17NO	192.1383	-2	5.99
12	C20H28N2	297.2325	-0.8	6.14
13	C20H28N2O	313.2274	0.6	6.24
14	C20H28N2	297.2325	-1	6.44
15	C20H28N2	297.2325	0.7	6.7
16	C23H25N	316.2060	-0.7	6.71
Methamphetamine	C10H15N	150.1277	-	3

Table 1. Showing all markers in 3 samples

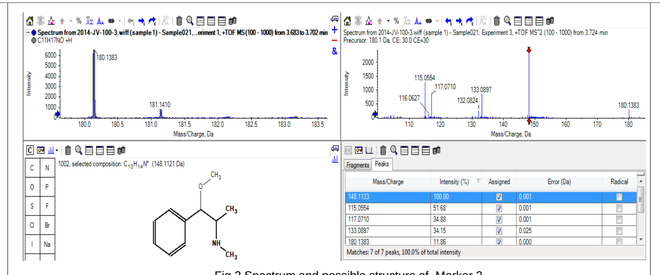

Fig.2 Spectrum and possible structure of Marker 2

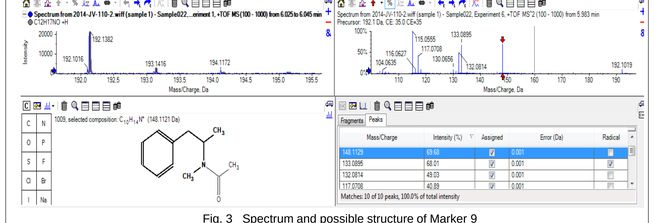

Fig. 3 Spectrum and possible structure of Marker 9

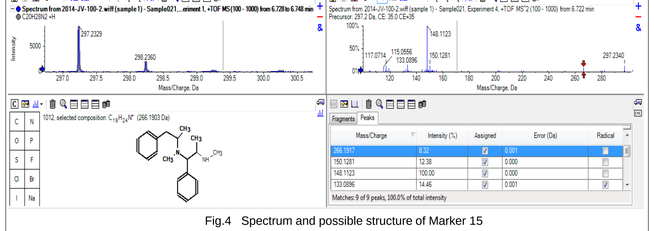

Fig.4 Spectrum and possible structure of Marker 15

Pearson correlation analysis of 9 samples from the seizure of a case

	S1	S2	S3	S4	S5	S6	S7	S8	S9
S1	1	0.990012	0.998377	0.973961	0.998764	0.99941	0.998429	0.998157	0.999026
S2		1	0.984841	0.98303	0.988024	0.992203	0.987242	0.993602	0.988799
S3			1	0.970425	0.999458	0.997043	0.999387	0.994976	0.999426
S4				1	0.973755	0.977341	0.973285	0.978872	0.974611
S5					1	0.998015	0.999579	0.996745	0.999892
S6						1	0.997867	0.999369	0.998365
S7							1	0.996704	0.999546
S8								1	0.997185
S9									1

Table 2 Pearson correlation analysis result

From the result table, sample S4 is much different from others.

CONCLUSIONS

An ultra-fast liquid chromatography coupled with hybrid quadrupole time-of-flight tandem mass spectrometry method which featured high resolution, mass accuracy and sensitivity, was developed to detect impurities for profiling in methamphetamine samples synthesized by Emde method. Sixteen impurities as markers were selected. Case to case comparison method was also successfully developed by Pearson correlation analysis of their relative contents in different seizures.

REFERENCES

1. Forensic Science International , 177 (2008), 153–161

TRADEMARKS/LICENSING

For Research Use Only. Not for use in diagnostic procedures.
The trademarks mentioned herein are the property of AB Sciex Pte. Ltd. or their respective owners.
AB SCIEX™ is being used under license.

© 2014 AB SCIEX.