Analysis of Cannabinoids and Amphetamines in Serum by RRLC / Triple Quadrupole Mass Spectrometry Using a Multimode Ion Source

Application

Toxicology

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Abstract

LC / MS / MS is a useful analytical technique for the analysis of amphetamines and cannabinoids in biological matrices. Amphetamines ionize well in electrospray ionization (ESI), whereas cannabinoids exhibit better sensitivity with atmospheric pressure chemical ionization (APCI). Using a 1.8-µm particle size RRHT column for the LC separation, the Agilent G1978B multimode ion (MMI) source was utilized in order to achieve a balanced response for both compound classes in a single analysis. The presented method exhibits good within-day and day-to-day reproducibility. The coefficients of variation ranged from 3 to 15%; most of the coefficients were in the 5 to 10% range.

Introduction

Cannabis is the most widely used illicit drug in Europe and in the United States of America. Furthermore, the abuse of sympathomimetic drugs such as amphetamine derivatives has increased considerably during the last years. Consequently, driving under the influence of drugs of abuse has become a serious problem for traffic safety. Due to this reason, driving after consumption of cannabis and amphetamines, including their methylenedioxy-derivatives methylenedioxymethamphetamine (MDMA) and methylenedioxyethylamphetamine (MDE), was sanctioned by the German Road Traffic Act in 1998. Since then, the number of toxicological analyses of serum for Δ9-tetrahydrocannabinol (THC) or amphetamine derivatives has increased enormously. Therefore laboratories need analytical methods that can handle a large number of samples in a relative short time. An appropriate technique to meet these needs is LC/MS/MS.

Amphetamines are basic polar compounds and ionize well in electrospray ionization (ESI), whereas the relatively nonpolar cannabinoids exhibit better sensitivity with atmospheric pressure chemical ionization (APCI) (Figure 1). To use the optimum ionization technique for each class of drug in a single run, the Agilent G1978B multimode ion (MMI) source (Figure 2) was evaluated in order to achieve a balanced response for both compound classes. The MMI source can operate in either ESI or APCI modes or in “mixed” mode, which is simultaneous ESI and APCI. The choice and parameters of ionization mode can be time-programmed during the run.

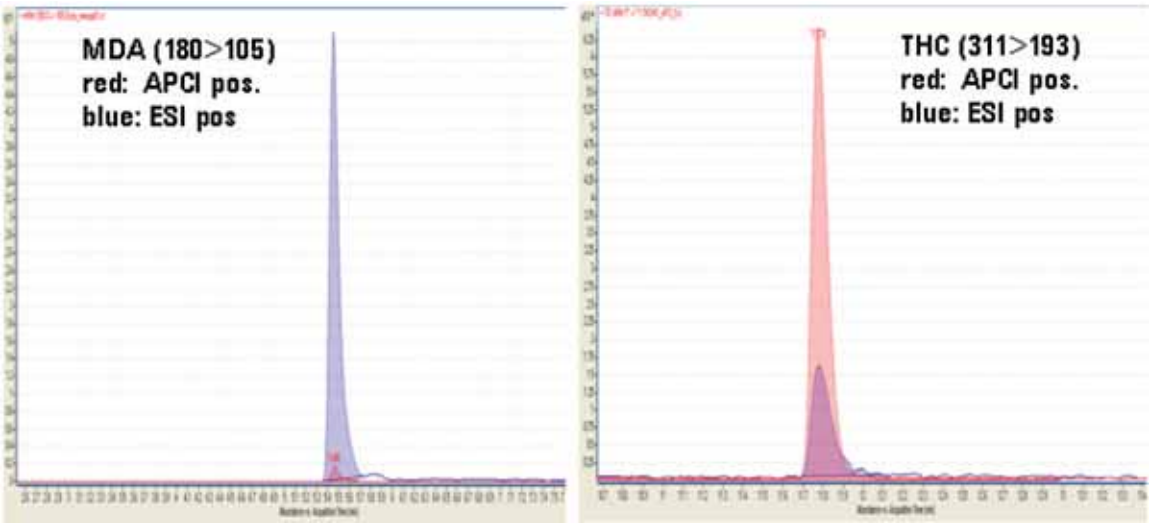

Figure 1. Comparison of the MDA and THC response in ESI and APCI modes.

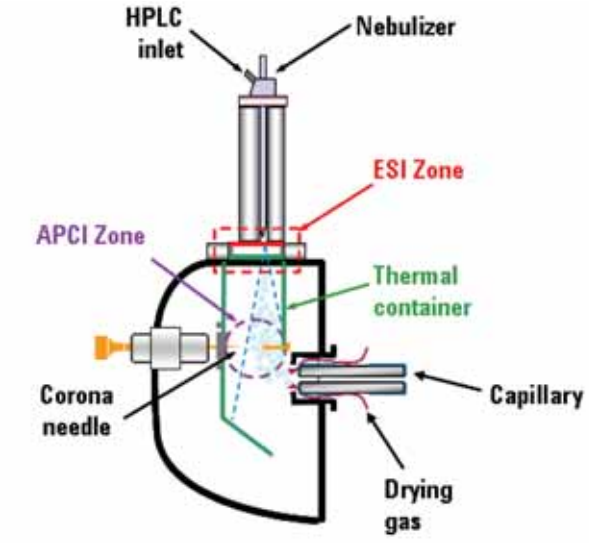

Figure 2. Design of the multimode source.

Experimental

Reagents

All solvents and reagents were analytical grade. Methanol, acetone, acetic acid, dichloromethane, 2-propanol, and ammonia were purchased from E. Merck (Darmstadt, Germany) or from Sigma-Aldrich (Deisenhofen, Germany). Solid-phase extraction columns were purchased from Mallinckrodt Baker (Griesheim, Germany), and all drug standard solutions and deuterated compounds were purchased from Cerilliant (Austin, TX).

Sample Preparation

A 1-mL sample of serum was diluted with 6 mL of phosphate buffer (0.1 M, pH 6). Then 50 µL of the internal standard mixture was added (1 ng/µL each of methanolic solution of D_{11}-amphetamine, D_{11}-methamphetamine, D_5-MDA, D_5-MDMA, D_6-MDE, and D_9-THC-COOH and 0.1 ng/µL each of D_3-THC and D_3-THC-OH). The sample was mixed for 3 minutes and the mixture was centrifuged at 3,000 rpm for 10 minutes. Solid-phase extraction was either automated, using the Caliper RapidTrace SPE workstation, or was done manually, using a vacuum manifold.

The supernatant was applied to a solid-phase extraction column (Bakerbond SPE C18, 500 mg), which had been conditioned by flushing with 2 × 3 mL of methanol and 2 mL of water. The column was rinsed with 2 × 2 mL water, 2 × 2 mL water/methanol (80:20; v/v), and 1 mL of 0.1 M acetic acid. The column was dried for 10 minutes.

The elution was carried out in two steps. First the cannabinoids were eluted with 3 mL of dichloromethane/acetone (50:50; v/v), followed by elution of amphetamines, opiates, and cocaine/metabolites with 3 mL of dichloromethane/2-propanol/ammonia (40:10:2; v/v/v). Both extracts were evaporated under a slight stream of nitrogen at 30 °C, reconstituded in 0.1 mL methanol, and added together. This combined SPE fraction was diluted with water (ratio 1:4) to improve the chromatographic peak shape.

LC / MS / MS Method

The LC/MS/MS consisted of an Agilent 1200 Rapid Resolution liquid chromatograph and an Agilent G6410A Triple Quadrupole mass spectrometer. Different ZORBAX columns were evaluated in combination with different solvents, flow rates, and column parameters to optimize the speed of the analysis while maintaining a good chromatographic resolution.

The best results were obtained with a 1.8-µm particle size ZORBAX SB-C18 column (2.1 × 100 mm) using a water/acetonitrile gradient (both containing 0.1% formic acid). The detailed LC conditions are listed in Table 1.

Table 1. LC Method

Column	Zorbax RRHT SB-C18 (2.1 mm id × 100 mm, 1.8 µm) p/n 828700-902
Column temperature	70 °C
Mobile phase	A: 0.1% formic acid in water B: 0.1% formic acid in acetonitrile
Flow rate	0.6 mL/min
Gradient	10% B at 0 min 10% B at 2 min 95% B at 8 min 95% B at 11min 10% B at 11.5min
Stop time	15 min
Post time	None
Injection volume	10 µL (sample diluted with water 1:4 to improve peak shape)

In addition to the standard ESI and APCI sources, an Agilent G1978B Multimode source was coupled to the mass spectrometer. The MMI source was operated in mixed mode (ESI and APCI simultaneously in positive polarity) or alternatively in pure ESI and APCI modes, switching between these ionization techniques based on a chromatographic time scale. The optimized source parameters are shown in Table 2.

Determination of the optimal MRM transitions for both analytes and internal standards was carried out by flow injection analysis of the single components at concentration levels around 1 µg/mL. See Table 3.

Results and Discussion

Four possible MMI modes were investigated (Figure 3). Using an MMI method that begins in ESI mode and switches to APCI mode after five minutes resulted in the best overall compound responses. The LC method was not fully optimized for speed (Table 1) because the vaporizer temperature is changed from 175 to 250 °C after the switch of the ionization mode, and that change requires some short time before the cannabinoids elute. The total run time, including the re-equilibration time of the column at starting gradient conditions, was 15 minutes.

Table 2. Optimized MMI-Parameters

MMI mode	Neb. press (psi)	Drying gas flow (L/min)	Drying gas temp (°C)	Charging voltage (V)	Capillary voltage (V)	Vaporizer temp (°C)	Corona current (µA)
Mixed	40	5	300	2000	2000	200	2
ESI	60	5	300	2000	2000	175	0
APCI	20	5	300	2000	2000	250	4

Table 3. Data Acquisition Parameters for the MRM Transitions

Compound	RT (min)	Precursor $(M-H)^+$	Frag (V)	CE (V)	Product ion (*m/z*)	CE (V)	Product ion 2 (*m/z*)
Amphetamine	1.3	136	100	15	91	10	119
D_{11}-Amphetamine	1.3	147	100	15	127	15	97
MDA	1.4	180	100	15	105	15	135
D_5-MDA	1.4	185	100	10	168	15	138
Methylamphetamine	1.5	150	100	15	91	10	119
D_{11}-Methylamphetamine	1.5	161	100	15	127	15	97
MDMA	1.9	194	100	10	163	15	135
D_5-MDMA	1.9	199	100	15	165	10	135
MDE	2.6	208	100	15	135	15	147
D_6-MDE	2.6	214	100	15	166	15	136
THC-OH	7.9	331	110	30	193	30	201
D_3-THC-OH	7.9	334	110	20	316	25	196
THC-COOH	8.1	345	110	30	193	30	299
D_9-THC-COOH	8.1	354	120	22	308	25	196
THC	8.6	315	110	30	193	30	259
D_3-THC	8.6	318	100	30	196	30	105

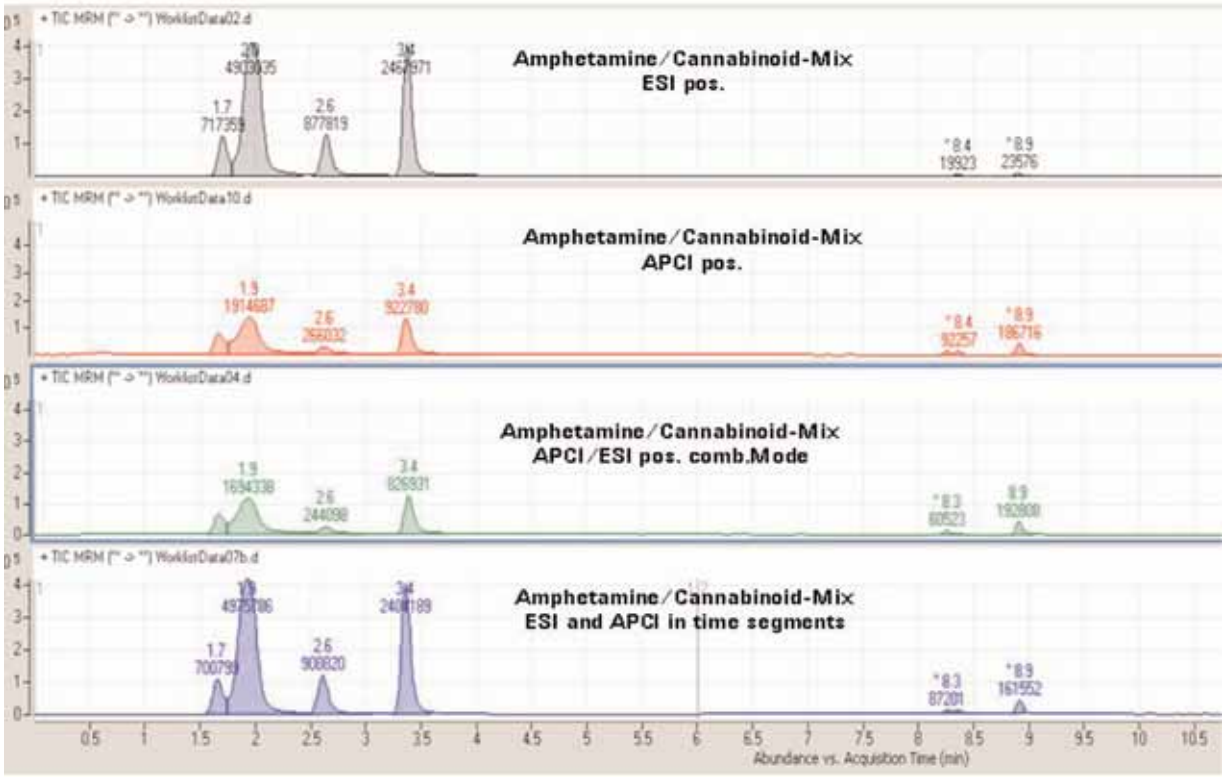

Figure 3. Comparison of the different MMI modes.

Method Validation

The LC/MS/MS method was validated for the detection and quantification of THC, THC-OH, THC-COOH, amphetamine, methamphetamine, MDA, MDMA, and MDE in serum. The validation of the method was carried out according to Peters et al [1] and the German Society of Toxicology and Forensic Chemistry (GTFCh). The method validation was performed by using a Microsoft Excel-based validation program (VALISTAT [2]). Drug-free serum was used as a blank matrix for the validation measurements.

Seven calibration standards were prepared. The different calibration levels were obtained by spiking the blank serum with 50 µL of methanolic solutions containing appropriate amounts of the analytes. The calibration levels are shown in Table 4.

Table 4. Calibration Range for Amphetamines and Cannabinoids in Serum Samples

Compound	Cal 1	Cal 2	Cal 3	Cal 4	Cal 5	Cal 6	Cal 7	Cal 8
Amphetamine	0	10	20	40	60	80	100	500
Methamphetamine	0	10	20	40	60	80	100	500
MDA	0	10	20	40	60	80	100	500
MDMA	0	10	20	40	60	80	100	500
MDE	0	10	20	40	60	80	100	500
THC	0	0.5	1	2	3	4	5	25
THC-OH	0	0.5	1	2	3	4	5	25
THC-COOH	0	5	10	20	30	40	50	250

A seven-point calibration curve for each compound was obtained by measuring of the calibration standards in six replicate injections. The calibrations were linear in the range tested and the correlation coefficients were > 0.98 for all compounds. The S/N calculations for calibration standard Cal 3, which represents the limit of quantitation, were based on peak-to-peak noise definition and no smoothing was applied. All quantifier and qualifier ions of the amphetamines and cannabinoids can be easily detected, even when diluting the methanol-reconstituted SPE fractions with water (ratio 1:4) to improve the chromatographic peak shape (Figure 4).

Intra-assay and inter-assay precision data were obtained from two analyses in a series performed on eight different days at two concentration levels (low, high). The intra-assay precision (within-day reproducibility) is defined as the mean value of the eight coefficients of variation (CV) from the two measurements carried out on one day. Inter-assay precision (day-to-day reproducibility) is the coefficient of variation from the average of the eight mean values of the two measurements carried out on one day. The intra-assay coefficients of variation ranged from 2.9 to 15.3 % (Table 5). The day-to-day coefficients of variation ranged from 3.4 to 15.3 %

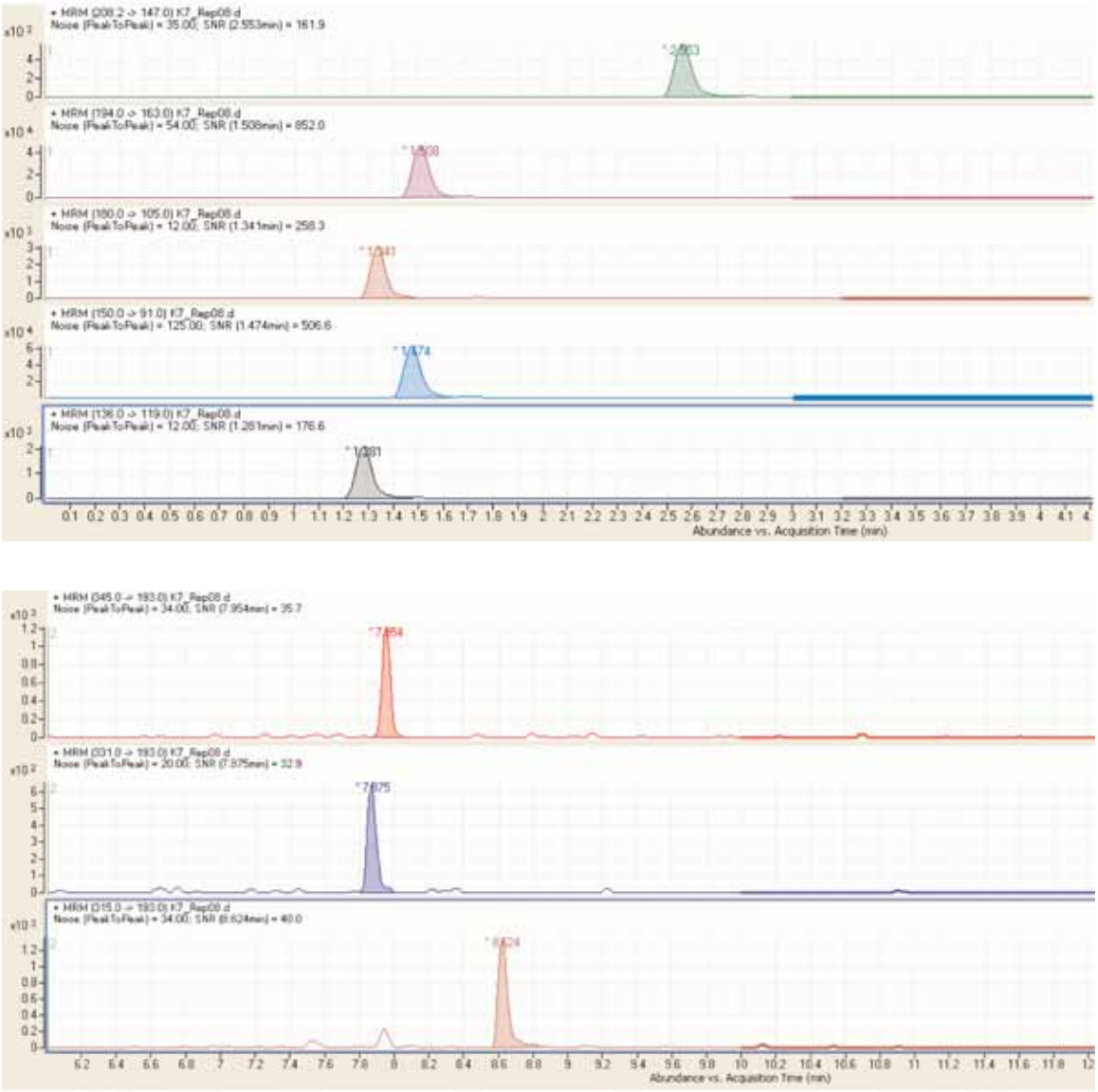

Figure 4. S/N calculation for standard Cal 3.

Table 5. Inter-Assay and Intra-Assay Precision at Two Concentration Levels (Cal 3 and Cal 7)

	Intra-assay precision in %		Inter-assay precision in %	
Compound	**CalStandard 3**	**CalStandard 7**	**CalStandard 3**	**CalStandard 7**
Amphetamine	4.3	2.9	4.9	5.6
Methamphetamine	4.7	4.7	6.1	5.5
MDA	7.6	4.2	8.4	4.6
MDMA	5.9	3.2	5.9	3.4
MDE	8.5	5.2	8.5	5.6
THC	9.5	5.8	10.0	6.3
THC-OH	8.4	8.7	11.6	8.7
THC-COOH	15.3	5.4	15.3	5.5

Conclusions

The use of a 1.8-µm particle size RRHT column for the LC separation provides a faster analysis (cycle time 12 min) than GC/MS (cycle time 45 min). Due to the polarity differences of the two compound classes, the use of the multimode ion source allows the detection of the eight compounds with an optimal response for each compound (switching the ionization mode on a time-based scale leads to the best results). In comparison to the established GC/MS method, the RRLC/QQQ method shows a higher sensitivity and selectivity (considering an injection volume of 1 µl in GC/MS and 10 µl in LC/MS/MS with a dilution factor of 4). The presented method exhibits good within-day and day-to-day reproducibility. The coefficents of variation ranged from 3 to 15%; most of the coefficients were in the 5 to 10% range.

In the future, other drugs of abuse (opiates like morphine, 6-acetylmorphine, and codeine as well as cocaine and its metabolites) will be included in this RRLC/QQQ method. Also, the use of online SPE will be evaluated.

References

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2. G. Schmitt, M. Herbold, and F. Peters, “ Methodenvalidierung Im Forensisch-Toxikologischen Labor,” Arvecon Walldorf (2003)

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