Validation of an HPLC Method for Quantitation of MDMA in Tablets

I.B. Müller* and **C.N. Windberg**

Department of Forensic Chemistry, University of Copenhagen, Frederik V's Vej 11, 2100 Copenhagen, Denmark

Abstract

An isocratic reversed-phase high-performance liquid chromatography (HPLC) method is developed and validated for the quantitation of 3,4-methylenedioxymethamphetamine (MDMA) in tablets. The chromatographic separation is achieved with potassium phosphate buffer (pH 3.2)–acetonitrile (9:1, v/v) as mobile phase, a Chromspher B column, and UV detection at 210 nm. The calibration curve is linear from 1.4 to 111 µg/mL. The percent relative standard deviation for intra- and interday precision studies is 2.7% each. The measurement uncertainty is estimated to 9%. The method is specific and successfully used for routine quantitation of MDMA in tablets.

Introduction

3,4-Methylenedioxymethamphetamine (MDMA) (Figure 1) or "ecstasy" is a commonly abused drug that has had a significant increase in popularity over the years. MDMA is a synthetic drug with both psychedelic and stimulant effects. The drug was synthesized in 1912 as a possible appetite suppressant drug. In the 1980s, however, MDMA entered the lists of internationally controlled substances (1). Currently, MDMA is predominantly a "club drug" and is commonly used at rave parties.

MDMA is a stimulant with additional psychedelic effects that may last between 4 and 6 h. MDMA is usually taken in oral tablet form. The psychological effects of MDMA include confusion, depression, anxiety, sleeplessness, drug craving, and paranoia. Adverse physical effects include muscle tension, involuntary teeth clenching, nausea, blurred vision, feeling faint, tremors, rapid eye movement, and sweating or chills. The risk of dehydration and hyperthermia increased by the excessive physical activity of dancing makes this combination a possible lethal cocktail (1). An increase in drug intoxications, some with mortal outcomes, in MDMA-related abuse has been observed in Denmark in 2000, alerting the Danish National Board of Health.

A national monitoring program conducted by the Danish National Board of Health in collaboration with the national police and the Departments of Forensic Chemistry (3 laboratories in Denmark) was established in 2001, and every seizure of illicit tablets has since been analyzed. The department has developed a method to separate MDMA from other derivates, such as amphetamine, *N*-ethyl-3,4-methylenedioxyamphetamine (MDE), and paramethoxyamphetamine (PMA). The method was validated to comply with specified requirements using the most recommended guidelines for analytical validation in Europe (2,3), including the most widely applied analytical-performance characteristics such as stability of the analytical solutions, selectivity, recovery, accuracy, precision, linearity, limit of detection (LOD) and quantitation (LOQ), ruggedness, and measurement uncertainty (4,5). The purpose of this study was to develop and validate a rapid, simple, accurate, and sensitive high-performance liquid chromatography (HPLC) method for the quantitation of MDMA in tablets. The results show that the method is applicable by the police.

Experimental

Chemicals and reagents

MDMA was purchased from Lipomed AG (Arlesheim, Switzerland). HPLC-grade acetonitrile was obtained from Rathburn (Microlab, Aarhus, Denmark). Potassium diphosphate (KH_2PO_4) was obtained from Merck (Darmstadt, Germany). Deionized water from Milli-Q plus ultrapure water system (Millipore, Billerica, MA) was used throughout the experiment.

HPLC instrumentation and conditions

The HPLC system consisted of an Agilent 1100 system with

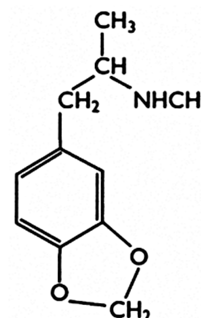

Figure 1. Chemical structure of MDMA.

* Author to whom correspondence should be addressed: email Irene.breum@forensic.ku.dk.

Reproduction (photocopying) of editorial content of this journal is prohibited without publisher's permission.

quaternary pump, autosampler, thermostated column oven, and diode array detector (Agilent Technologies, Copenhagen, Denmark). The data were acquired by Chemstation software (Agilent Technologies). For HPLC instrumentation see Table I. Chromatograms of MDMA gave good symmetrical peak shapes (Figure 2), and the spectra comparison curve fit was more than 900 using a in-house generated spectrum library.

Table I. HPLC Instrumentation Parameters

Instrumentation	Parameters
Column	Chromspher B with 100% deactivated C18 material (5 µm, 100- × 3-mm) Chrompack (Varian, Vaerloese, Denmark)
HPLC solvent (mobile phase)	0.05M phosphate buffer pH 3.2–acetonitrile (9:1, V:V)
Diode array detector	210 nm* (spectrum comparison at 205–400 nm)†
Flow rate	0.4 mL/min
Oven temperature	30°C
Injection volume	20 µL

* The nonspecific lower wavelength 210 nm was chosen because it is necessary to detect other compounds that could be in the powders.
† Identification was confirmed by a UV-curve fit of 900 or more and peak purity of 990 or more.

Table II. Examination of Recovery (Estimated to 100%)

Theoretic sample conc. (µg/mL)	Weight (mg) 1	Weight (mg) 2	Conc. found (µg/mL)	Recovery (%)
0 (N = 2)	47.9	52.2	0	–
3.1 (N = 2)	51.4	52.3	2.90	93.4
15.5 (N = 2)	50.0	49.7	15.75	101.6
31.0 (N = 2)	51.1	51.6	30.80	99.3
61.9 (N = 2)	51.7	60.1	60.32	97.4
68.0 (N = 2)	51.8	51.4	66.62	98.0

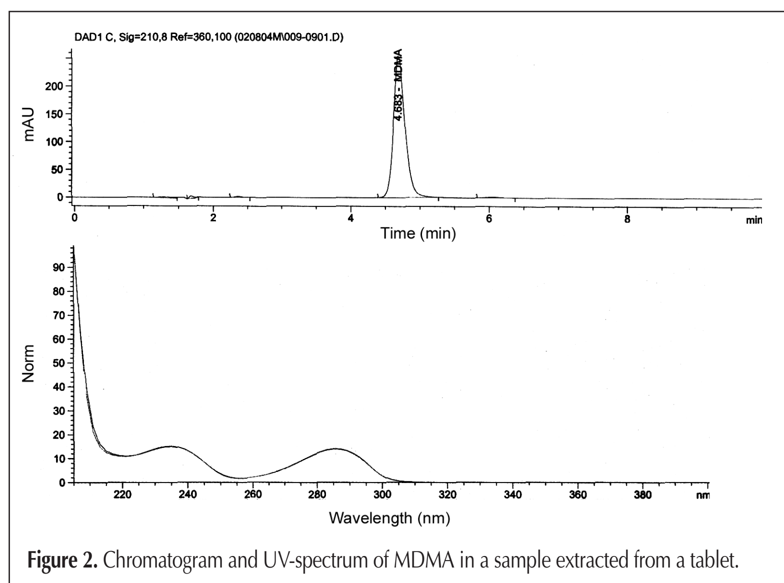

Figure 2. Chromatogram and UV-spectrum of MDMA in a sample extracted from a tablet.

Calibration standards and quality control samples preparation

Samples were quantitated using a five-point calibration curve: 1.4, 2.5, 12.3, 22, and 111 µg/mL MDMA in HPLC solvent. The calibration standards were prepared by diluting in-house stock standard solution (1000 µg/mL) in HPLC solvent.

To make the test material resemble the unknown matrix constituents (such as lactose or glucose), quality control samples were prepared from an illicit batch of MDMA tablets (26 mg MDMA in 100 mg powder) at 62 µg/mL. For the validation experiment, test samples were prepared at six concentration levels (0, 3.1, 15.5, 31.0, 61.9, and 68.0 µg/mL) from the control batch material. For every routine run, one quality control sample (62 µg/mL) was analyzed in duplicate, and the results were plotted on a quality control chart.

Sample preparation

A tablet was homogenized using a mortar and pestle. To 50 mg of powder, 10.0 mL HPLC solvent was added. The mixture was shaken for 5–10 min and subsequently centrifuged for 10 min at 3000 *g*. The sample was filtrated using a syringe filter (0.5 µm) (Frisennette Apo, Ebeltoft, Denmark) and diluted using 50-µL sample extract in 1 mL HPLC solvent, giving the dilution factor (F), and 20 µL was injected into the HPLC system. No internal standard was added because it was estimated that the recovery of the extraction was 100% (Table II). The contentration of MDMA in one tablet was calculated using the following equations:

$$\text{MDMA in \%} = \left[\frac{(\text{concentration in sample [µg/mL]} \times F \times 10\text{ mL} \times 100\%)}{(\text{weight [µg]})}\right] \quad \text{Eq. 1}$$

$$\text{MDMA in mg} = \left[\frac{(\text{MDMA [\%]} \times \text{weight of tablet [mg]})}{100}\right] \quad \text{Eq. 2}$$

Sampling of tablets

Prior to MDMA analysis, representative sampling of the seizure must be performed before further analysis. Visual inspection and weighing are used to establish if a seizure is heterogeneous or homogenous. When there are more than 150 tablets, a representative sample of 150 tablets are inspected. If there are visual differences, 300 tablets have to be investigated. If less than 300 tablests, all of the tablets have to be investigated. If the number of tablets is less than 150, all tablets are investigated visually, 10 tablets are investigated by shape and size, and 2 tablets are randomly selected, homogenized, and analyzed using HPLC.

Results and Discussion

Validation of the method

The following validation parameters were examined and are summarized in Table III.

Stability of the analytical solutions

Samples and solutions were chromatographed immediately after preparation. The quality control samples (0.062 mg/mL) were reassayed after storage at 5°C for 32 days. The results showed no significant change in MDMA concentration (less than 5%) over the examined period.

Selectivity

The possible interference of similar compounds was investigated, including amphetamine, ephedrine, metamphetamine, phentermine, MDE, PMA, paramethoxymetamphetamine (PMMA), and the co-component caffeine. The selectivity of the method was determined by a spectral purity check of each compound. The acceptance criteria were a purity of 990 or more. The results confirmed the specificity of the method, in which only PMMA coeluted with MDMA. Samples containing PMMA were identified by preliminary gas chromatography–mass spectrometry screening, and a mixture of PMMA and MDMA was analyzed using another HPLC method. Examination of a possible lack of response using authentic samples (tablets) containing either one, or mixtures of lactose and glucose, showed no interference and confirmed the specificity of the method.

Accuracy

The accuracy of the method, ratio of the determined concentration, and nominal concentration were determined by the analysis of two external quality control samples supplied by proficiency test supplier European Network of Forensic Science Institutes (ENFSI) (6). The results showed that the *z*-scores were 0.49 and 1.09, respectively, and both results were in the ± 5% window. Furthermore, a batch of illicit tablets, used as the quality control test sample, was analyzed by three other laboratories (two in Denmark and one in Sweden). The test results gave a percent relative standard deviation (%RSD) of less than 2%.

Precision

The precision of the method was measured as intra- and interday precision. Intraday results were obtained by injecting six replicate extractions during the same day. The test was conducted at six concentration levels on two HPLC systems. Interday precision was investigated using a random design at six concentration levels (4 replicates) on six different days with four samples extracted and injected each day. The test was performed by three technicians and on two HPLC systems, with both parameters randomly selected. The results were evaluated by standard statistical methods. The %RSD values for both the intra- and interday were estimated to 2.7% and illustrated a good precision of the analytical method.

Table III. Method Validation Results

Validation steps	Parameters	Results
Stability of the analytical solutions	Quality samples were analyzed after storage at 5°C from 1–32 days	There were no significant changes (< 5%) in MDMA concentration in this period
Selectivity	The selectivity was determined by spectral purity check. Possible interference of similar compounds* was checked	Peak putity was found to be 990 or more and UV-curve fit of 900 or more. Only PMMA coeluted with MDMA
Accuracy	Two quality control samples (ENFSI) and an illicit sample used for the validation were analyzed by three different laboratories	The *z*-scores from the two quality control samples were 0.49 and 1.09. The % RSD of the three different laboratories was less than 2 %
Precision	Intraday (one single day) – 6 levels 4 times. Interday (6 different days) – 6 levels each†	The %RSD for both intra- and interday calculation was 2.7%
Linearity	Linearity was calculated using the precision tests data	Follow equations was found: $y = 1.0x - 0.45$ the peak area y versus concentration x expressed in µg/mL. The correlation factor was $r^2 > 0.99$
LOD and LOQ	LOD and LOQ were based on the precision tests data‡	LOD = 1 µg/mL LOQ = 3 µg/mL
Ruggedness	Changes that might occur during routine analyses, (Table 4).	Changes in pH, solvent composition, column batch, flow, and extraction volume were possible without significant influence
Measurement uncertainty	Module-based uncertainty evaluation system was used (8)	$CV_{results}$ = 9%

* Amphetamine, ephedrine, metamphetamine, MDE, phentermine, PMA, PMMA and caffeine
† Demonstrated by three analysts using two HPLC systems and the results evaluated across the two HPLC systems.
‡ The calculated LOD and LOQ were verified by preparing MDMA samples in lactose matrix in the found concentration.

Linearity

The results of the precision test design were used to calculate the linearity and the following equations were found by plotting peak area (y) versus concentration (x) expressed in µg/mL. The equations from the two HPLC systems are combined and simplified to the following:

$$y = 1.0x - 0.45 \quad \text{Eq. 3}$$

A correlation factor of > 0.99 was achieved. Correction of data is omitted because corrected and uncorrected data showed only minor acceptable variations.

LOD and LOQ

The LOD and LOQ were estimated using the precision test design values. The estimated LOD and LOQ were verified by preparing MDMA in a lactose matrix using HPLC analysis. The LOD was 1 µg/mL and LOQ was 3 µg/mL.

Ruggedness

The ruggedness of the chromatographic method was examined by changing parameters in the system that might be affected during routine

analysis, such as the temperature of the column oven, column batch, solvent composition (buffer–acetonitril), pH in the buffer, flow rate, UV wavelength, and extraction volumes (Table IV). Alternative levels for the parameter's solvent composition, column oven temperature, pH in buffer, and flow rate were selected to investigate the flexibility of the method to changes intentionally applied to improve for chromatographic separations of unknown mixtures of compounds in future seizures (7). Changes in column batch, solvent composition and pH, flow, and extraction volume were possible with a standard deviation (SD) of less than 5%. The method was less robust to changes in UV detection and temperature of the column oven, which both caused a SD of more than 5% of the test result. Control of both factors is currently performed according to in-house standard operational procedures.

Measurement uncertainty

To calculate the measurement uncertainty, the module-based uncertainty evaluation system was used (8). This method consists of four main steps; specification, identification, quantitation, and combination. The specification step proposes the relationship between the measurement and treatment and the properties it depends upon. It could concern a specific property of a sample, such as the stability of the sample, environmental influence, etc. The identification step identifies possible sources of uncertainty, it could be the uncertainty caused by subsampling from nonhomogeneous samples. The quantitation step expresses the uncertainties as standard uncertainties. The combination step combines the standard uncertainties and calculates the total uncertainty.

Following model equation was used:

$$CV_{result} = \sqrt{CV_{ana}^2 + CV_{cal}^2 + CV_{pre}^2} \quad \text{Eq. 4}$$

where CV_{result} was the estimated uncertainty. CV_{ana} (uncertainty of the analytical analysis) was estimated from the quality control charts over a period of half a year (5.4%). CV_{cal} (uncertainty of the calibrators) was estimated from two uncertainty contributors [the uncertainty of the purity of the component MDMA (0.14%) used and the uncertainty of the "in-house" made calibrator solutions (1.0 %)]. CV_{pre} (uncertainty of the preanalytical preparation and analysis) was estimated by examination of the uncertainty of subsampling from nonhomogeneous samples, preparation of the solution, and the analysis on HPLC (7.5%); this value was estimated using both inter- and intraday measurements. The measurement uncertainty CV_{result} was calculated to 9%.

Table IV. Robustness Parameters of the HPLC Method

Factors	Nominal	Alternative 1	Alternative 2
Temperature (column oven) (°C)	30	25	35
Column (batch)	1	2	2
Solvent composition (buffer–acetonitril)	(9:1, v/v)	(9.5:0.5, v/v)	(8:2, v/v)
pH in buffer	3.2	3.0	3.5
Flow rate (mL/min)	0.4	0.6	0.3
UV wavelength (nm)	210	205	215
Extraction volume (mL)	10.0	9.8	10.2

Conclusion

An HPLC method for quantitation of MDMA was developed and validated. The results showed that the method is selective, as only one compound, PMMA, coelueted with MDMA. The linearity and accuracy were confirmed, and the precision and measurement uncertainty was acceptable. The ruggedness tests showed that moderate changes in the column, solvent composition and pH, flow rate, and extraction volume had no effect on the results, while changes in the column temperature and UV wavelength had to be controlled. The method was used for quantitation of MDMA in tablets and powder.

Acknowledgments

The authors would like to thank the technicians Birgitte Drehn, Hanne Hvenegaard, and Vibeke Christiansen for the practical work.

References

1. Office of National Drug Policy. MDMA (ecstasy). *Microgram* **XXXV(6):** 149–56 (2002).
2. The Commission of the European communities. Commission decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation. *Off. J. Eur. Comm.* **L221:** 8–36 (2002).
3. Eurachem. *CITAC: Quantifying Uncertainty in Analytical Measurement*, 2nd ed., 2000, http://www.eurachem.ul.pt/guides/QUAM2000-1.pdf.
4. ICH. ICH Harmonized Tripartite Guideline. Text on Validation of Analytical Procedures. In proceedings of *International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human use*. 1994, http://www.ich.org/MediaServer.jser?@_ID=417&@_MODE=GLB.
5. F.T. Peters and H.H. Maurer. Bioanalytical method validation and its implications for forensic and clinical toxicology – a review. *Accred. Qual. Assur.* **7:** 441–49 (2002).
6. European Network of Forensic Science Institutes (ENFSI) WG Drugs Collaborative Study, Netherlands Forensic Institute, Ministry of Justice, Amsterdam, the Netherlands (Proficiency test supplier).
7. W.J. Youder and E.H. Steiner. *Statistical Manual of the Association of Official Analytical Chemistry.* AOAC, Washington, D.C., 1975, pp. 33–36.
8. J. Kristiansen. Description of a generally applicable model for the evaluation of uncertainty of measurement in clinical chemistry. *Clin. Chem. Lab. Med.* **39(10):** 920–931 (2001)

Manuscript received December 6, 2004;
revision received June 3, 2005.