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Stability study of the designer drugs "MDA, MDMA and MDEA" in water, serum, whole blood, and urine under various storage temperatures

Karine M. Clauwaert, Jan F. Van Bocxlaer, André P. De Leenheer*

Laboratory of Medical Biochemistry and Toxicology, Ghent University, Harelbekestraat 72, B-9000 Gent, Belgium

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Abstract

A controlled study was undertaken to determine the stability of the designer drugs MDA, MDMA and MDEA in pooled serum, whole blood, water and urine samples over a period of 21 weeks. The concentrations of the individual designer drugs in the various matrices were monitored over time, in the dark at various temperatures (−20, 4 or 20°C), for a low (±6 ng/ml for water, serum and whole blood and ±150 ng/ml for urine) and a high concentration level (±550 ng/ml for water, serum and whole blood and ±2500 ng/ml for urine). Compound concentrations were measured using a validated HPLC assay with fluorescence detection. Our study demonstrated no significant loss of the designer drugs in water and urine at any of the investigated temperatures for 21 weeks. The same results were observed in serum for up to 17 weeks, and up to 5 weeks in whole blood. After that time, the compounds could no longer be analyzed due to matrix degradation, especially in the low concentration samples that were stored at room temperature. This study demonstrates that the designer drugs, MDA, MDMA and MDEA are stable when stored at −20°C for 21 weeks, even in haemolysed whole blood.

Keywords: Stability study; Designer drugs; 3,4-Methylenedioxymethamphetamine; 3,4-Methylenedioxyethylamphetamine; 3,4-Methylenedioxyamphetamine

1. Introduction

The designer drugs MDMA (3,4-methylenedioxymethamphetamine, ecstacy), MDEA (3,4-methylenedioxyethylamphetamine, eve), and MDA (3,4-methylenedioxyamphetamine), the latter being a designer drug but also an important metabolite of both MDMA and MDEA, are all methylenedioxyamphetamine derivatives whose use has increased dramatically by the mid 1980s [1]. Though, generally regarded as relatively safe recreational drugs, it has become apparent that the use of methylenedioxyamphetamine derivatives can be associated with severe adverse effects sometimes leading to a fatal outcome [2–5].

As such, the designer drugs are a group of compounds which are frequently analyzed in toxicological laboratories (both clinical and forensic). Knowledge of the stability of a drug is of importance for toxicologists in several situations, limitations of a logistic nature often introduce variable time intervals between sampling of the matrices and analysis. Moreover, when the time period between the death of a subject and the sampling of the matrices is significant, chemical degradation, besides, ongoing enzymatic metabolisation and redistribution, potentially becomes an issue. Even in these kind of situations, the toxicologist should be able to determine if interpretation of the obtained quantitative data can be performed reliably. Nevertheless, little knowledge, except for one study where the stability of MDA and MDMA during 47 days in frozen plasma was investigated [6], can be found regarding the stability of MDA, MDMA and MDEA in biological matrices. Our study aims to provide such information and complete the available (bio-)analytical data on MDA, MDMA and MDEA.

For the determination of the designer drugs in our stability study, we used a previously published method [7] based on

* Corresponding author. Tel.: +32-9-264-8131; fax: +32-9-264-8197.
E-mail address: andre.deleenheer@rug.ac.be (A.P. De Leenheer).

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HPLC analysis with detection through the native fluorescence of the methylenedioxylated amphetamines. It uses liquid–liquid extraction after appropriate internal standardization with a suitable MDMA analogue, "MDMPA" or "methylenedioxymethylpropylamphetamine". To our knowledge, this is the first investigation of the stability of MDA, MDMA and MDEA in several biological matrices at different temperatures.

2. Materials and methods

2.1. Apparatus

The HPLC unit was composed of a ternary low pressure gradient pump, and autosampler with a 25 μl loop (Kontron Instruments, Milano, Italy) equipped with a solvent degassing module (Shodex, Tokyo, Japan). A spectrofluorometric detector (RF-10Axl; Shimadzu, Kyoto, Japan) linked to a Kromasystem 2000 data system (Kontron Instruments) was used for data acquisition and storage.

2.2. Reagents and materials

All reagents and chemicals were of analytical grade and were from Aldrich (Gillingham, UK) unless stated otherwise. Solvents were of HPLC grade from Fisher Scientific (Loughborough, UK) or Merck (Darmstadt, Germany). MDA, MDMA and MDEA pure standards were from Sigma (St. Louis, MO) and the internal standard (IS) MDMPA was synthesized by us following a procedure described earlier [7]. The stock solutions, containing 1 mg of compound per milliliter methanol were stored in the dark at −20°C and were stable for at least 1 year. Working solutions were stored under the same conditions as the stock standards but discarded after 6 months.

2.3. Isolation of the compounds

250 μl of water, serum and whole blood or urine samples (stability study, blank or calibration samples) were extracted with 8 ml of hexane/ethylacetate (7/3, v/v), after addition of 50 μl of the IS solution (containing 20 ng MDMPA in 50 μl for water, serum and whole blood; and 250 ng in 50 μl for urine), dilution with 1 ml of H_2O and adjustment of the pH to 9.5 with 1 M aqueous K_2CO_3. Samples were mixed on a rotatory mixing device (10 min) and centrifuged for 15 min (1200 × *g*). The organic layer was transferred to a test tube containing 50 μl methanolic HCl (5 M acetylchloride in methanol) and evaporated using a Turbovap® evaporator (Zymark, Hopkinton, MA) at 35°C under nitrogen. The residue was redissolved in 100 μl (water, serum and whole blood) or 250 μl (urine) HPLC eluent A (see Chromatography sections) and a 25 μl aliquot was injected for liquid chromatographic separation with fluorescence detection.

2.4. Chromatography

Chromatographic separation was achieved on a Hypersil BDS C_{18} column (100 mm × 2.1 mm, 3 μm, Alltech, Deerfield, IL). The mobile phase was a 0.1 M solution of ammonium acetate in HPLC-grade water (90%), methanol (5%) and acetonitrile (5%) (eluent A) or in methanol (45%), acetonitrile (45%) and HPLC-grade water (10%) (eluent B). After an isocratic part (100% A) of 6 min, a linear gradient from 0 to 70% B within 14 min was used.

2.5. Fluorescence detection

The excitation and emission wavelengths of the fluorescence detector were 288 and 324 nm, respectively, (bandwidth was 15 nm for both slits).

2.6. Calibration samples

Calibration curves ranging from 2 to 1000 ng/ml (water, serum and whole blood) and from 100 to 5000 ng/ml (urine) and were prepared in the corresponding blank matrix. The calibration samples were all extracted according to the general isolation procedure.

2.7. Stability samples

For each matrix (serum, whole blood, water and urine), two pools containing all three compounds were prepared. A pool containing a low concentration (±6 ng/ml for serum, whole blood and water and ±150 ng/ml for urine) which is called the low concentration stability sample and a pool at a high concentration level, the high concentration stability sample (±550 ng/ml for serum, whole blood and water and ±2500 ng/ml for urine) were divided in 1 ml aliquots in glass tubes. The concentrations used were chosen on the basis of concentrations found in real postmortem as well as clinical cases analyzed in our laboratory. The obtained aliquots were placed in appropriate containers, protected from light and stored at −20, 4 or 20°C. To determine the exact concentrations and the precision of the method, an extra set of eight samples was aliquoted and stored at −20°C. These samples were analyzed by two different operators, the first four consecutive days of our stability study. For the stability study itself, one aliquot of each matrix for every storage condition was analyzed at the different time points (at the start of the study, after 1, 3, 5, 9, 13, 17 and 21 weeks) of the study.

3. Results and discussion

3.1. Recovery of the compounds

For the determination of MDA, MDMA, MDEA and the internal standard, a simple, inexpensive and robust single-step extraction procedure was developed. The

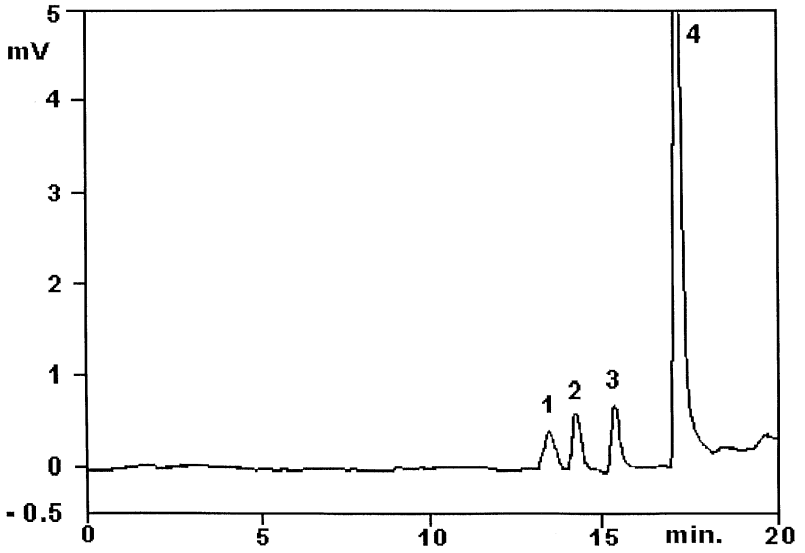

Fig. 1. Chromatogram of a low concentration stability serum sample, ±6 ng/ml for MDA (1), MDMA (2) and MDEA (3), and 80 ng/ml of the internal standard, MDMPA (4).

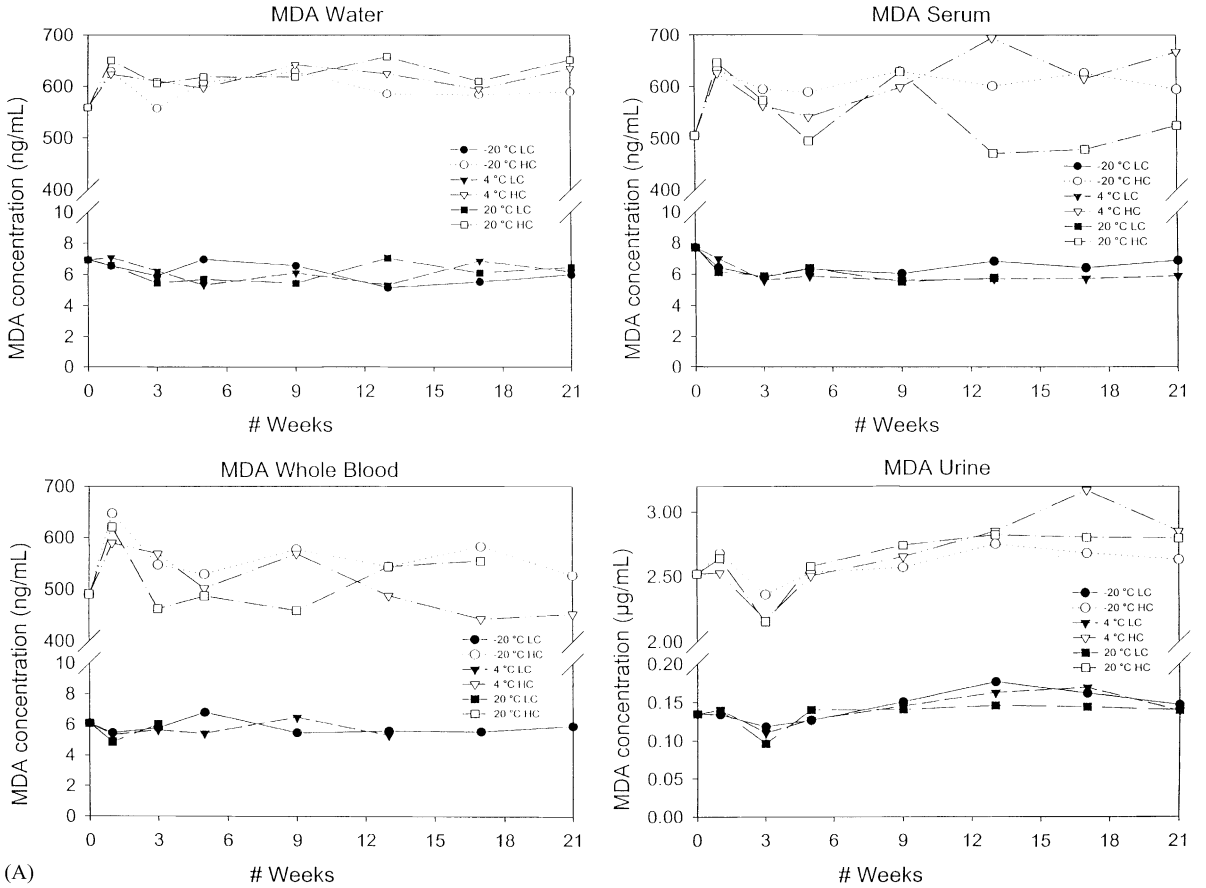

Fig. 2. Stability data for MDA (A); MDMA (B) and MDEA (C). LC: low concentration stability sample, and HC: high concentration stability sample.

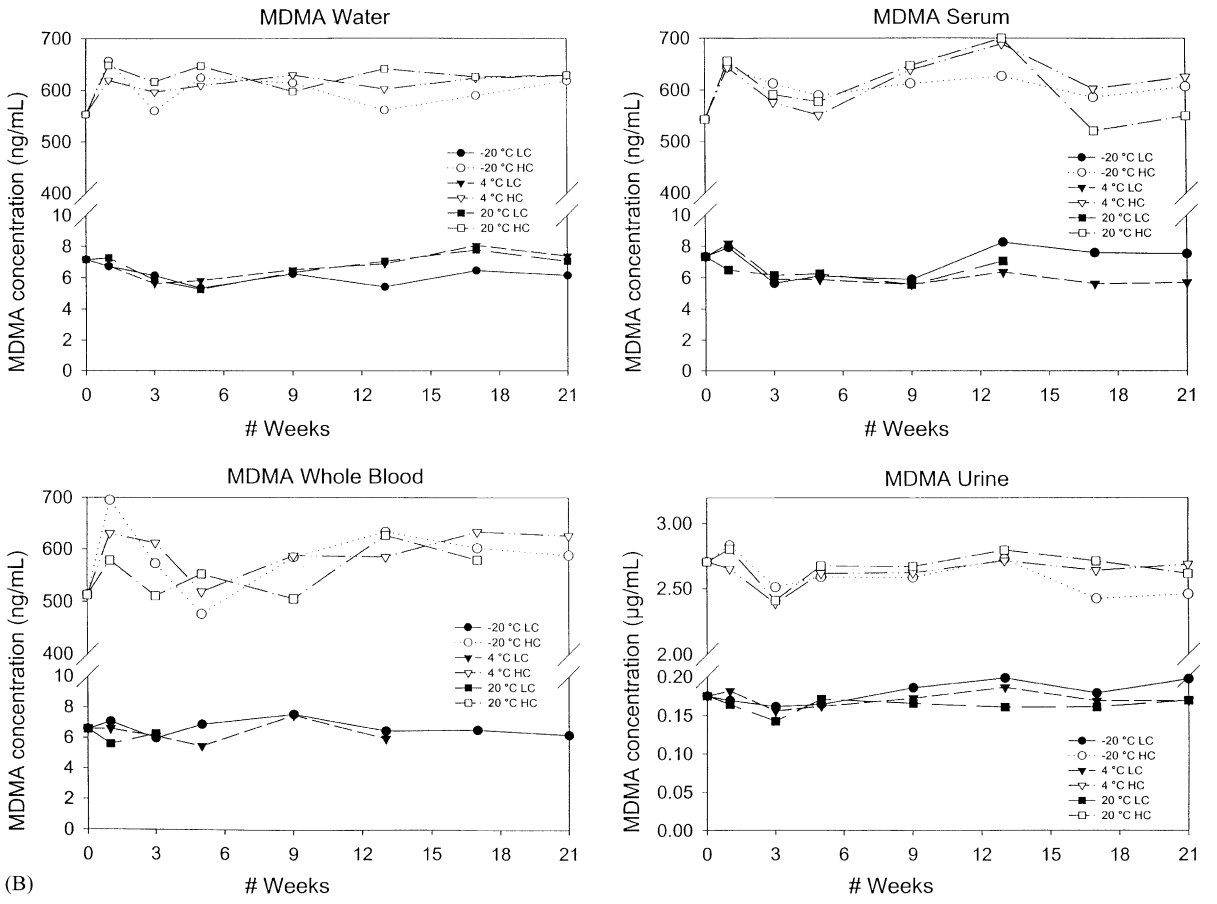

Fig. 2. (*Continued*).

obtained extraction recovery results were above 70% and indicate that the analyzed compounds can effectively be extracted with hexane/ethylacetate (70/30, v/v) at pH 9.5 from the various biological matrices.

3.2. Chromatography

Using the HPLC conditions described, MDA, MDMA and MDEA are well resolved (resolution >1.5) and elute in symmetrical peaks. The retention times of MDA, MDMA, MDEA and the IS are 13.2, 14.0, 15.3, and 16.9 min, respectively, yielding capacity factors (k') of 7.0; 7.5, 8.3, and 9.3. Fig. 1 shows the chromatogram of an extract of an analyzed serum sample (stored at 4°C for 13 weeks). As can be seen from this chromatogram, there were no interfering peaks present from endogenous compounds. This, however, changed for serum stored at room temperature for 17 weeks and whole blood stored at room temperature for 5 weeks. After this period, proper identification and integration of the peaks were, especially for the low concentrated samples, impossible due to the presence of a high amount of background fluorescence originating from matrix degradation products. The same phenomenon was found for the low concentrated whole blood samples that were stored for longer than 13 weeks at 4°C.

3.3. Validation of the analytical method

The method was extensively validated [7] according to the now in bioanalytical applications accepted validation criteria. The following is a summary of the obtained results. The method proves linear ($r^2 > 0.997$) over a range of 2–1000 ng/ml for water, serum and whole blood and 100–5000 ng/ml for urine. The method shows extraction recoveries >70%, good precision (2.5–18.9%) and accuracy (95.5–104.4%). The L.O.D. is 0.8 ng/ml and the L.O.Q. is 2 ng/ml in water, serum and whole blood and 2.5 ng/ml and 0.1 µg/ml, respectively, for urine.

3.4. Stability data

The obtained stability data are shown in Fig. 2. Visual inspection of this figure already reveals that for MDA, MDMA and MDEA, no particular degradation can be observed in the measured samples. Indeed, no downward trend can be found in the concentration points, as analyzed in

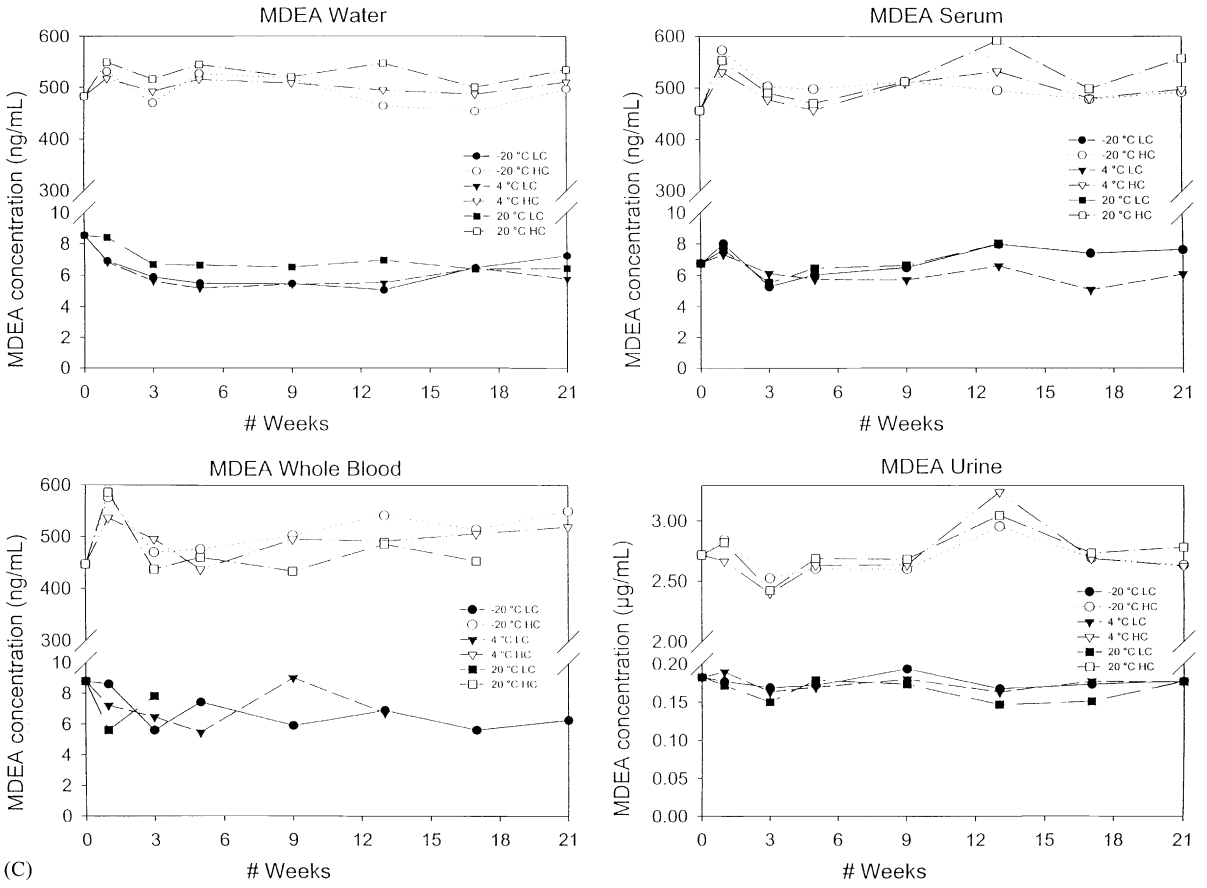

Fig. 2. (*Continued*).

all four matrices. This is also proven by the data given in Table 1. At the start of our stability study, the separately aliquoted samples (for every matrix, for both concentration levels) were analyzed over 4 consecutive days by two different operators to establish the samples exact concentration and the precision of the analytical method at those particular concentrations. The mean concentrations found (data of the first 4 days, $n = 8$), together with the obtained precision data (CV%) of the method can now be compared with the mean concentrations and the precision data obtained for the whole set of stability samples (subdivided by matrix and concentration level) analyzed over 21 weeks at the three storage temperatures ($n = 24$, if all samples can be analyzed, not the case for whole blood, low concentration: $n = 17$; serum, low concentration: $n = 22$; whole blood, high concentration: $n = 23$). If no difference can be found comparing the mean concentrations, we can positively conclude that no degradation occurs. Statistical data analysis was applied to test this. To that end, we performed an *F*-test to compare the variances. If the *P*-value (Table 1) obtained for this test is <0.05, the difference between the variances is judged significant. This was only the case for three data sets: the low concentration MDEA water samples, and the low concentration MDMA and MDEA whole blood samples. In all cases, except the latter three, a *t*-test, assuming equal variances could be performed. In the latter cases, we used the corresponding *t*-test not assuming equal variances. The values of *P* obtained for the *t*-test of the data sets are given in Table 1. Since, no $P < 0.05$ was obtained for all 24 analyzed data sets we can conclude that the differences between the means of the immediately analyzed, separately aliquoted samples and of the complete stability samples data set (21 weeks, 3 stored temperatures) are not significant. As a result, we can draw the same conclusion as in the visual test of Fig. 1 that no degradation of MDA, MDMA and MDEA takes place when water, serum, whole blood and urine samples are stored at −20, 4 and 20°C, except for the complication that low concentration samples, more particular whole blood and serum sample, stored at room temperature for long periods (5 weeks for whole blood and 17 weeks for serum) could no longer be analyzed due to interference from matrix degradation. The same has to be concluded for low concentrated whole blood samples stored at 4°C for periods longer than 13 weeks. However, it is clear that the compounds themselves are very stable chemical entities, requiring no particular attention in a great variety of (bio-)chemical conditions.

Table 1
Mean concentrations and coefficients of variation of the method (values obtained the first 4 days of the stability study) and of the complete data set obtained over 21 weeks at all three tested temperatures with the statistical data analysis values obtained when tested to compare variances and means (HC: high concentration stability sample, LC: low concentration stability sample)

	Water			Serum			Whole blood			Urine		
	MDA	MDMA	MDEA	MDA	MDMA	MDEA	MDA	MDMA	MDEA	MDA	MDMA	MDEA
LC												
Mean concentration (ng/ml)	5.81	5.99	5.73	6.42	7.39	6.81	5.39	6.55	7.14	136	175	169
Coefficient of variation of the method	10.3%	6.43%	14.5 %	10.1%	10.7 %	4.14%	6.62%	3.75 %	7.91%	9.59%	4.72%	9.88%
Mean concentration (ng/ml)	6.21	6.55	6.55	6.33	6.61	6.65	5.76	6.46	7.13	140	171	172
Coefficient of variation over 21 weeks	10.2 %	11.7%	16.3%	11.0%	13.7%	13.4%	8.12%	8.77%	18.1%	10.3%	7.20 %	6.78%
F-test for variances, *P*-value	0.984*	0.094	0.566	0.930	0.769	0.009[a]	0.522	0.048[a]	0.049[a]	0.888	0.329	0.200
Student's *t*-test for means, *P*-value	0.154	0.077	0.076	0.764	0.054	0.584[a]	0.078	0.596[a]	0.984[a]	0.616	0.381	0.595
HC												
Mean concentration (ng/ml)	605	577	487	579	602	509	540	560	478	2500	2657	2826
Coefficient of variation of the method	5.43 %	9.39%	7.92%	9.00%	7.36%	7.59%	8.13%	7.30%	5.97%	2.50%	3.65%	2.82%
Mean concentration (ng/ml)	608	607	506	579	603	503	529	575	491	2626	2633	2709
Coefficient of variation over 21 weeks	5.03%	5.23%	5.17%	10.8%	8.72%	7.45%	10.4%	9.51%	9.07%	8.22%	4.58%	6.68%
F-test for variances, *P*-value	0.728	0.059	0.167	0.679	0.869	0.826	0.599	0.484	0.272	0.128	0.617	0.384
Student's *t*-test for mean, *P*-value	0.800	0.071	0.135	0.998	0.942	0.707	0.640	0.517	0.480	0.155	0.643	0.124

* $P < 0.05$ means that difference is significant (two-tailed test).

In all cases except [a]equal variances could be assumed (*F*-test), in [a]cases *P*-value of *t*-test not assuming equal variances is given.

4. Conclusion

We have conducted a thorough investigation of the stability of MDA, MDMA and MDEA under various storage temperatures in the dark. We found that no degradation occurred at any of the investigated temperatures over a period of 21 weeks. The results obtained by Garrett et al. [6] who reported unchanged MDA and MDMA concentrations in frozen plasma after a shorter period of 1.5 months perfectly agree with our observation. However, especially for the low concentration stability samples in whole blood and serum, stored at room temperature, severe matrix degradation took place. As a result, the compounds could no longer be measured due to the presence of interfering compounds. However, at a low temperature (refrigerator or freezer), MDA, MDMA or MDEA containing samples (even the low concentration samples) can be stored for several weeks (13 weeks for whole blood and $\geq$21 weeks for the other matrices, water, serum and urine) without problems. Consequently, within the specified time limitation, no special precautions need to be taken, except the usual cooling of the samples, when MDA, MDMA and MDEA presence is suspected in biological samples which can not immediately be analyzed.

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