Short report validation (V077-16-11)

Determination of concentration of MDMA in pills or solids (powder/crystals) including detection of misdeclaration MDA, PMA/PMMA amphetamine/methamphetamine and 2C-B

Content

Introduction

The name MDMA refers to the active chemical ingredient: 3,4-methylenedioxy-N-methylamphetamine. The active ingredient content of MDMA pills is to be considered individually, because on the unregulated market there are pills with the same appearance (compression and color) but very clear differences in the MDMA content.

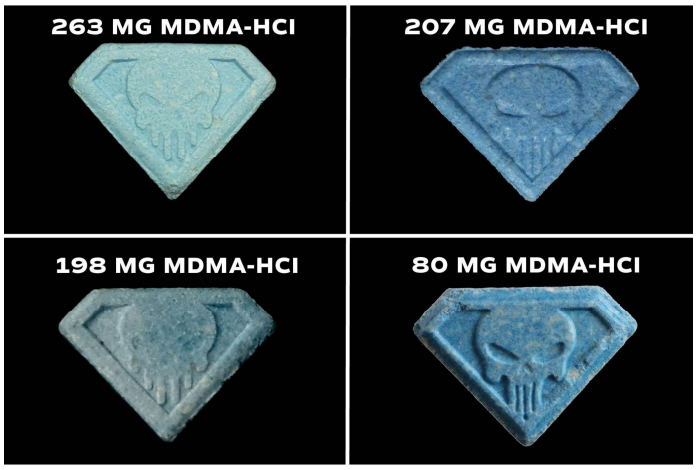


Figure 1: Same optical appearance with large differences in active ingredient concentration

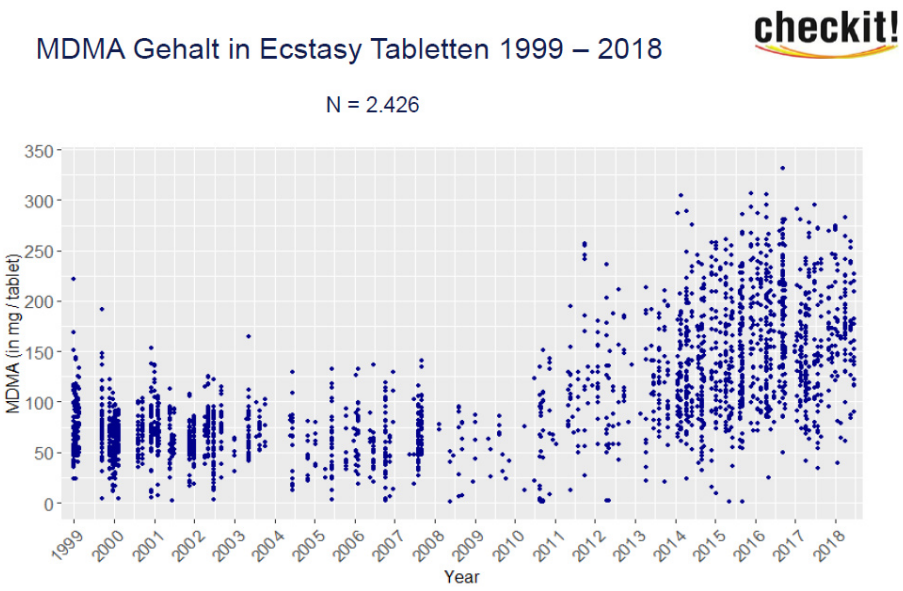


Figure 2: MDMA content of ecstasy tablets (n=2426) measured over the period 1999-2018; Source: Checkit Wien (Drugchecking Service in Austria).

The above graph (Figure 2) of the analysis data from the Austrian drug checking project CheckIt Wien illustrates the trend towards ever higher doses in tablets, with greater dispersion of the concentration levels at the same time. Based on a large amount of data over a long period of time, this shows the absolute necessity of measuring MDMA concentrations. The results of the analysis also show that a measurement method should be able to determine concentrations in the range of 50 - 300 mg MDMA in tablets. Ecstacy tablets are available in a wide variety of colors and forms, so a suitable test method must be robust against the influence of added color pigments and fillers for tableting (such as sugar, lactose, cellulose).

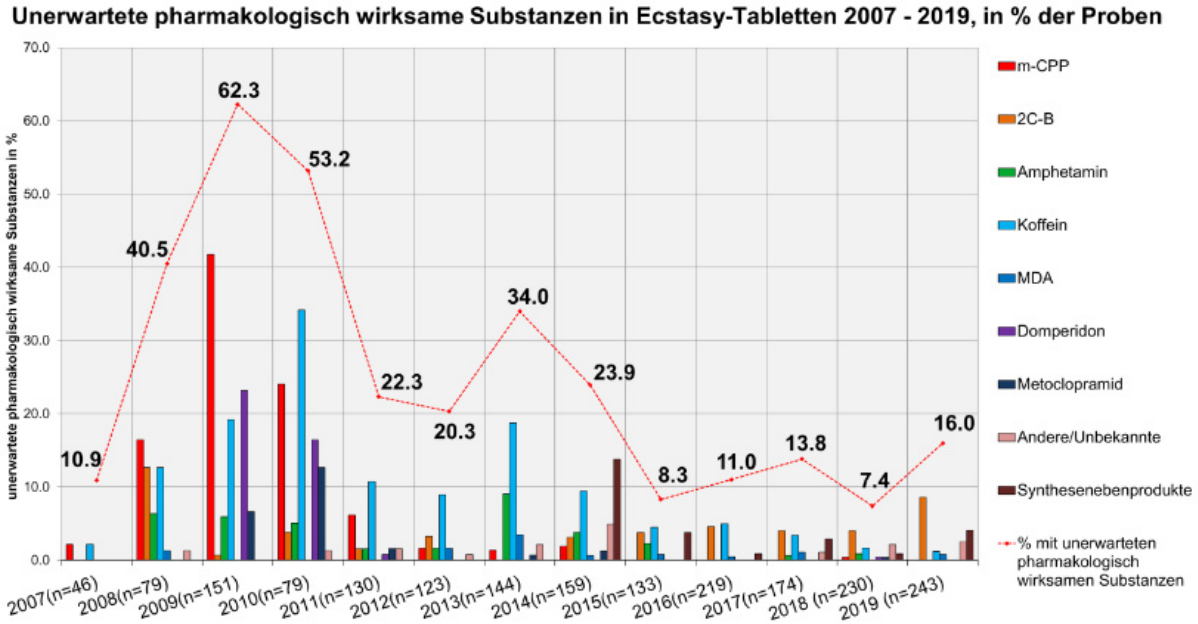


Figure 3: Unexpected pharmacologically active substances in ecstasy tablets from 2007-2019 in % of the samples, Source: saferparty.ch (government drug checking in Switzerland).

MDMA is also available in powder form or as a crystalline substance in which consumers cannot draw any conclusions about the actual presence of the active ingredient purely from the appearance of the substance. Here, misdeclarations cannot be ruled out. In addition, there is the problem of extenders and unexpected pharmacologically active substances, as shown in Figure 3. The trend clearly shows a decline in this problem, but confusion with 2C-B in particular still occurs frequently. Rarely, admixtures or mix-ups with MDA and amphetamine/coffeine occur. Figures 2 and 3 reflect the general current European trend of increasingly pure and high-dose pills in the market, with simultaneous high possible dispersion of the active ingredient concentration.

The miraculix MDMA QTest method

With the MDMA-QTest method, the concentration of the active ingredient MDMA can be analyzed within 15 minutes using an easy-to-use and mobile rapid test. The rapid test can be used to analyze not only crystalline or powdered pure substances, but also filled and colored ecstasy pills thanks to the 2-phase extraction. Even classic fillers such as lactose or cellulose do not interfere with the color reaction. The test procedure is divided into a two-phase extraction, from the lower phase of which the extracted active ingredient is taken by means of an enclosed pipette. This is placed in a large glass vial for concentration measurement and in two smaller vials for further analysis for misdeclaration. After adding the diluent, the result can be read off in all vials after 12 minutes by means of a color change.

The basis for the functionality lies in a linear colorimetric chemical reaction of MDMA with the detection reagent. This provides the basis for the first quantitative tests (Q-tests) for a wide variety of active ingredients. By means of the enclosed color chart, the tests can be easily evaluated by eye. Correctly performed tests (with white background and under daylight) achieve a high precision for a color rapid test. In specific, this means that inexperienced test persons only misjudge by a maximum of one color field when evaluating by color chart. It was found that comparison with reference photos of the entire color series significantly simplified the evaluation. A spectrophotometric evaluation achieves an even higher precision of the results (~5% deviation from the HPLC analysis).

It is a voluntarily validated and standardized test procedure based on the pharmaceutical method validation guideline ICH Q2(R1), which has been extended to the QTests. This guideline describes the requirements of the analytical method for active substances that are subsequently to be used in human medicine, and guarantees a safe dosage of these active substances, for example in pharmacies.

The MDMA-QTest was developed at the Friedrich-Schiller-University Jena at the Institute of Pharmacy, Chair of Pharmaceutical Microbiology, under the funding reference 03EGSTH1189, supported by the German Federal Ministry of Economics and Climate Protection as well as the European Social Fund, in the course of the third-party funded project "Production of quantitative test systems for psychotropic active substances" in the period from 2019 to 2021 under the project management of Dr. Felix Blei. The necessary permission for the handling and acquisition of the narcotics MDMA, MDA, 2C-B, amphetamine, PMA/PMMA and others

according to § 3 of the German Narcotics Act was granted for the Friedrich Schiller University Jena under the current BtM number 463 23 75 as well as previous for the Pharmaceutical Microbiology facility in Jena. The pure substances required for the project were purchased from LIPOMED GmbH and LGC Standards Ltd.

Summary

This extract from the validation summarizes the results of the quantitative test procedure for the concentration determination of MDMA in compressed tablet form or as a crystalline/powdered solid. The aim of the validation is to demonstrate the suitability of the MDMA QTest as a rapid test for the concentration determination of MDMA in pills and "powders". In addition, the suitability for the detection of 2C-B, amphetamine/methamphetamine, MDA, PMA/PMMA is to be investigated. The test system is a combined extraction and purification with a subsequent determination of the concentration by means of a color test.

Criteria for acceptance and test parameters

Parameter	Description and expected values	Acceptance criteria
Suitability test of the method of quantitative measurement of MDMA (linearity).	Dilution series with MDMA Reference: Stock solution MDMA 8mg/ml (in extraction solution) rising concentrations which mathematically represent the values of 4, 8, 12, 16, 20, 24, 28, 32 mg/ in 50mg sample material provide increasing color intensities, measurable in the spectrophotometer at 567nm and by eye	Dilution series with MDMA Reference: stock solution MDMA 8mg/ml (in extraction solution) n=3 Linear reaction with minimum or higher Pearson correlation coefficients R of 0.95
Suitability test of the colorimetric method for measuring colored pills	3 different colored MDMA pills are homogenized and 50 mg sample amount is analyzed according to MDMA QTest instructions.	Test with 3 different colored MDMA pills Despite dyed and filled pills a colorimetric color reaction purely to MDMA
Correctness of the method	Sample: MDA standard solution Sample: PMA/PMMA standard solution Sample: 2C-B standard solution Sample: Amphetamine HCL standard solution Sample: MDMA standard solution spiked with 2CB (30% and 50%) Sample: MDMA standard solution spiked with amphetamine	Sample: MDA standard solution Color reaction similar to MDMA (blue-purple) Sample: PMA/PMMA standard solution No color reaction with the detection solution Sample: 2C-B standard solution Color reaction in green color Sample: Amphetamine-HCL standard solution Color reaction in yellow/brown staining Sample: MDMA standard solution spiked with 2CB (30% and 50%) Clear recognition of a substance mixture based on color Sample: MDMA standard solution spiked with amphetamine Clear recognition of a mixture of substances by color
Accuracy and linearity of MDMA QTest Extender detection A+B	Sample: MDMA, MDA, 2-CB, PMA/PMMA Standard Solution Standard solution 8mg/ml	Sample: MDMA, MDA, 2-CB, PMA/PMMA Standard Solution Positive reaction for extender detection in the red lid for MDA, PMA and 2C-B (negative control) Positive reaction for extender detection in the blue lid for MDMA, PMMA (positive control)

Specificity of the method	Reagent blank Extraction solution in detection reagent is incubated	Reagent blank (n=3) Concentration MDMA: No MDMA detectable
Precision of the method	Repeatability Measure the 8 concentrations of the dilution series in the plate reader 3 times in succession, determine the standard deviation. Internal laboratory precision Measurement of the 8 concentrations of the dilution series in the plate reader on 2 different days by different analytes Measurement of the same dilution series on a different spectrophotometer	Repeatability No significant standard deviation with at least 3 concentrations x 3 replicates. Internal laboratory precision No significant standard deviation when measured by second analyte Equally linear curve when measured by second spectrophotometer
Detection and limits of determination	Limit of quantification LOQ by visual inspection Mixtures corresponding to 1, 2 , 3 , 4 mg MDMA in 50 mg sample material	Limit of quantification LOQ by visual inspection (n=3). Precision and accuracy ensured at lowest point of color evaluation scale (4mg MDMA/50mg substance).
Working range of the test procedure	Standard curve The performed experiments on the linearity of the measurement procedure define the working range, as expected the working range is due to the color evaluation scale of 4 - 32 mg MDMA per 50 milligram substance sample	Standard curve For the method of determining the uniformity of the content, the working range should normally cover 70% to 130% of the test concentration.
Robustness of the test procedure	Variation of incubation time for staining Only roughly crushed substance sample Variation extraction time	Variation of incubation time with staining Same staining with doubling of incubation time Only roughly crushed substance sample Same staining when sample material is only roughly crushed Variation extraction time Same staining when extraction time is doubled

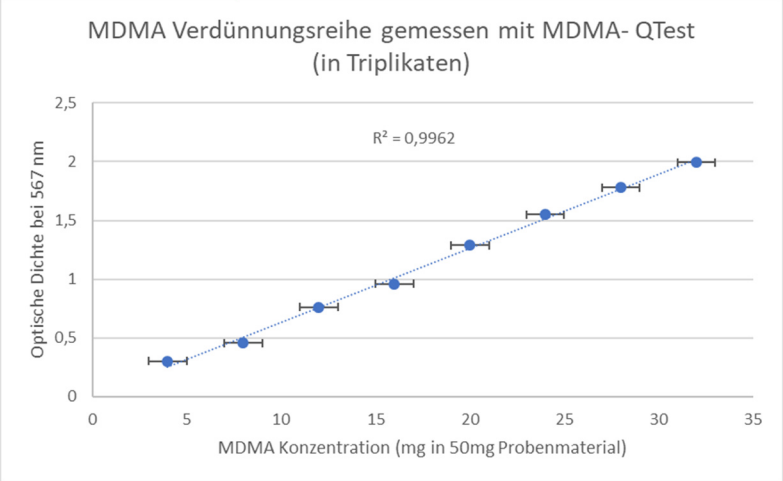
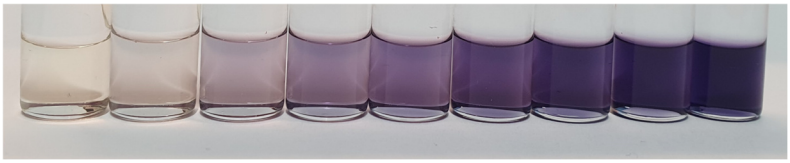
Methods

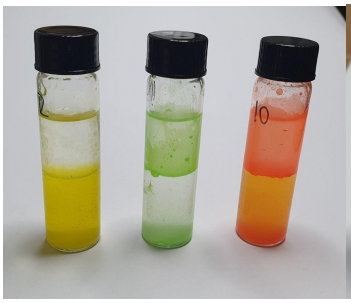
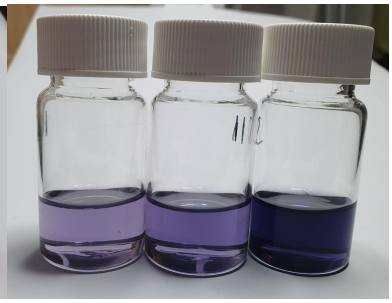
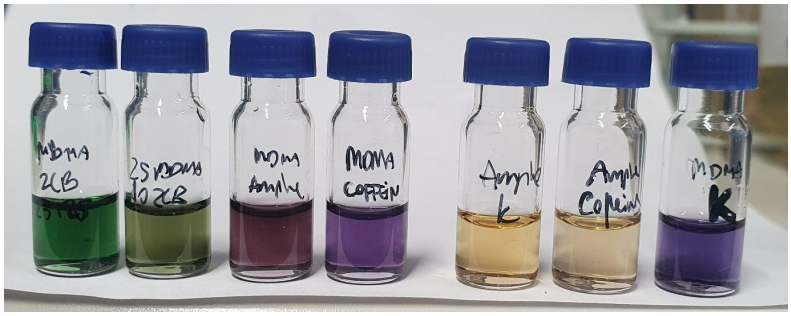
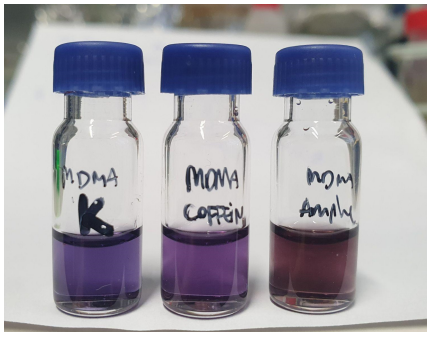
All measurements were performed at room temperature (21 °C), i.e. samples and reagents had an equally corresponding room temperature. The tests were performed according to the instructions enclosed with the rapid test set, and the corresponding deviation is marked in the methods.

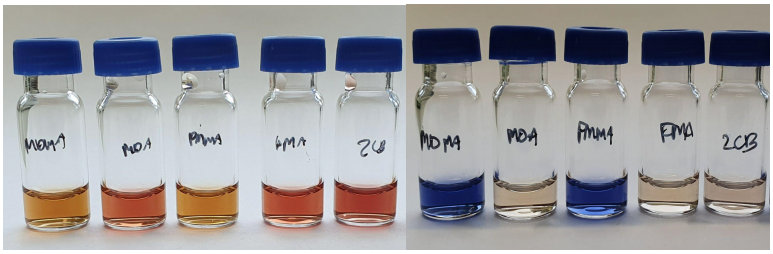
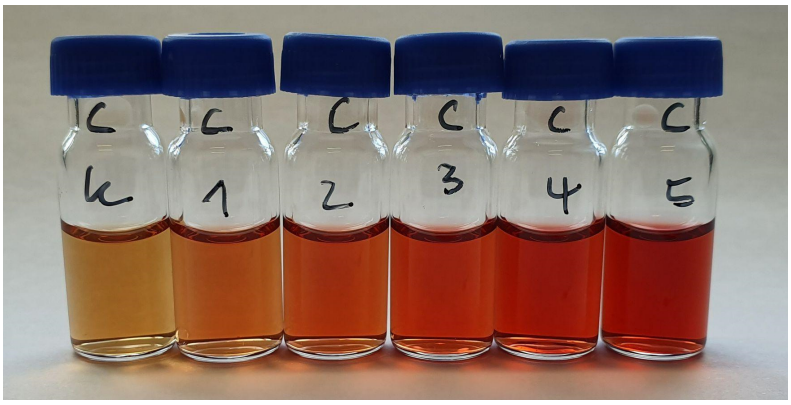
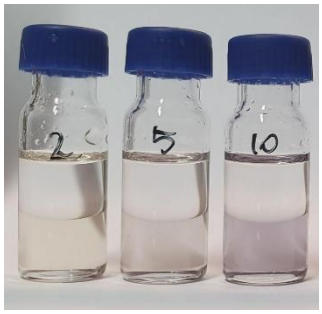
Sample	Optical validation MDMA QTest with MDMA pure substance: MDMA 8 mg/ml stock solution (in extraction solution lower phase)	Dilution series with MDMA Reference: stock solution MDMA 5 mg/ml (in extraction solution lower phase) Measurement at 567nm in plate reader	Reagent blank	Limit of quantification LOQ by visual inspection
PSB content	9,375 µl 18,75 µl 28,125 µl 37,5 µl 46,87 µl 56,25 µl 65,56 µl 75 µl	2 µl 4 µl 6 µl 8 µl 10 µl 12 µl 14 µl 16 µl	Extraction solution lower phase = Blank measurement	2 µl stock solution (5 mg/ml) in extraction solution 5 µl stock solution (5 mg/ml) in extraction solution 10 µl stock solution (5

				mg/ml) in extraction solution
Sample quantity	75 µl	16 µl	75 µl	10 µl
Extraction volume	ad 75 µl	16 µl	4 ml	ad 10 µl
Detection solution	6,50 ml	650 µl	6,5 ml	650 µl
Amount	n = 1	n = 3	n = 3	n = 1

Results

Sample name	**Results**	**Validation result**
Dilution series with MDMA Reference: stock solution MDMA 5+8 mg/ml (in extraction solution)	The spectrophotometer measurement showed a markedly linear chemical response with Pearson correlation coefficients R of 0.99. This is very close to a perfect result and is absolutely within the acceptance criteria of validation. Standard curve with compensation line to determine the correlation coefficient:  optical evaluation standard series with MDMA standard:  The optical evaluation of the dilution series showed a linear color gradation which can be arranged in an ascending row purely by eye without labeling.	**Validation passed**

Suitability test of the colorimetric method for measurement of colored pills	  A yellow, green and red/orange tablet were used for the test and carefully analyzed according to the test instructions. Despite the intensively colored tablets, the color result showed the expected coloration in all three tests, which could be quantitatively evaluated in the next step. The robustness is due not only to the purification but also to the fact that only 3 drops (50 µl) of the extraction solution are added to the almost 7 ml of detection reagent. Even colored phases as shown in the picture had no influence on the purple MDMA staining. In addition, classic fillers such as lactose and cellulose were mixed with MDMA pure, extracted/purified according to instructions, and also showed no influence on the MDMA staining.	**Validation passed**
Correctness of the method	 For the test, stock solutions of the substances 2C-B, amphetamine and MDMA were prepared and analyzed with the MDMA QTest. As described in the color evaluation, there was a clear green coloration for 2C-B (vial 1), even in the mix with MDMA (MDMA:2C-B, 70:30), a clear green coloration was already achieved which, based on the coloration, certainly indicates an impurity. With pure amphetamine, a yellow/orange coloration was seen, and the distinct purple coloration of MDMA shifts in the expected purple direction when both substances are mixed. Even amphetamine stretched with caffeine was still able to produce a distinct color reaction for amphetamine  Caffeine in MDMA also showed a clearly perceptible color difference which, however, was not as easy to detect without a control sample as the right MDMA sample with amphetamine admixture.	**Validation passed**

Accuracy and linearity of MDMA QTest Extender detection A+B	 For the validation of the integrated extender detection, stock solutions of the substances MDMA/MDA, PMA/PMMA, and 2C-B were prepared and extracted and analyzed according to the test instructions. The result was a clear reaction of MDA, PMA and 2C-B with the negative control (vials with red lids). In contrast, MDMA and PMMA showed no reaction with the detection reagent as expected. The positive control (vials with blue lids) showed staining with MDMA as well as PMMA, and no reaction with the MDA, PMA or 2C-B stock solution. For validation of a quantitative detection/LOD, a dilution series of MDA was prepared and analyzed by MDMA QTest. There was a clear gradation at the different concentrations, which also suggests a quantitative detection. 	**Validation passed**
Reagents blank	Result: No MDMA detectable, neither optically by eye nor by spectrophotometer measurement.	**Validation passed**
Limit of quantification LOQ by visual inspection	 Samples with very low active ingredient contents were measured for analysis of the quantitative limit of quantification. Starting (from left to right): 2 µl, 5 µl, 10µl MDMA stock solution (5 mg/ml). Even in the lowest concentration, a discoloration was already visible to the eye in comparison with the reagent blank (not shown in the photo). By measuring the reference standard, we know that the measurement method already delivers linear results in these ranges. So even well below the specified measuring range, the MDMA QTest already provides valid results.	**Validation passed**
Working range of the test method	By measuring the standard series with the spectrophotometer, it was possible to accurately characterize the linear range of the measurement procedure, which is far above the working range of the measurement procedure. In the optical comparison, the test persons (n=6) were also able to precisely analyze samples in the entire working range (4mg - 32mg in 50mg sample quantity). Just over half of the test persons were able to determine the correct concentration in the blind test, with just under 40% of the test persons estimating by only one color field. The active ingredient content of an average MDMA tablet weighing 0.5 g can thus be reliably analyzed in the range of 40 - 320 mg. This is clearly within the scatter range of the analyses of European ecstasy pills.	**Validation passed**

Robustness of the test procedure	The variation of the incubation time was doubled, no significant differences in the coloration were found in the comparison. Only roughly crushed substance samples took significantly longer until all components were dissolved in the extraction vessel, but subsequently showed the same color reaction. Doubling the extraction time also showed no significant changes in the indicated drug concentration.	**Validation passed**

Evaluation

The tested MDMA QTest by miraculix is safely suitable for the quantification of MDMA in tablets as well as solids. The experiments have shown the linear relationship of the optical density as a function of the MDMA concentration present over the entire measurement range. The results are directly proportional to the concentration present in the sample and therefore meet the necessary validation guidelines. The analyses were performed on different days and by different analytes, and proved to be absolutely accurate in these experiments. It is a thoroughly robust test method, which delivers absolutely reliable measurement results even when incubation or extraction times are extended or when only roughly crushed crystalline substances are used (with a resulting longer extraction time). The very low detection limits are also striking; a detectable discoloration of the detection reagent was already seen from 2 mg active substance content in 50 mg sample material. The working range of the test method is between 4 - 32 mg per 50 mg sample material due to the included color chart for evaluation and thus covers all available MDMA pill concentrations. Since MDMA tablets cannot contain more than ~ 60% of active ingredient due to the compression process, the MDMA-QTest covers the entire possible range in MDMA pills. If crystalline MDMA is present, it is sufficient to use only 30 mg of the substance, and a quantitative result (0 - 100% active ingredient content) is also obtained.

In the validation, the MDMA Q test method was shown to be easy to handle and perform, as well as fast and reliable in the evaluation. All acceptance criteria of the validation plan were met. The method is suitable for determining the concentration of MDMA in tablets or solids.