3D-printed portable device for illicit drug identification based on smartphone-imaging and artificial neural networks

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Abstract

In this manuscript, a 3D-printed analytical device has been successfully developed to classify illicit drugs using smartphone-based colorimetry. Representative compounds of different families, including cocaine, 3,4-methylenedioxy-methamphetamine (MDMA), amphetamine and cathinone derivatives, pyrrolidine cathinones, and 3,4-methylenedioxy cathinones, have been analyzed and classified after appropriate reaction with Marquis, gallic acid, sulfuric acid, Simon and Scott reagents. A picture of the colored products was acquired using a smartphone, and the corrected RGB values were used as input data in the chemometric treatment. ANN using two active layers of nodes (6 nodes in layer 1 and 2 nodes in layer 2) with a sigmoidal transfer function and a minimum strict threshold of 0.50 identified illicit drug samples with a sensitivity higher than 83.4 % and a specificity of 100 % with limits of detection in the microgram range. The 3D printed device can operate connected to a rechargeable lithium-ion cell portable battery, is inexpensive, and requires minimal training. The analytical device has been able to discriminate the analyzed psychoactive substances from cutting and mixing agents, being a useful tool for law enforcement agents to use as a screening method.

Keywords: drug classification, portable analytical devices, smartphone-based colorimetry, deep learning algorithms, colorimetric tests, screening procedures.

1. Introduction

According to the World Drug Report of the United Nations Office on Drugs and Crime (UNODC) in 2023 [1], drug use continues to be an important problem worldwide. The estimated number of drug users in 2021 is 296 million, 23 % more than in 2011. The UNODC estimated that by 2021, 36 million people had used amphetamines, 22 million had used cocaine, and 20 million had used "ecstasy"-type substances. Cocaine seizures have also grown significantly, reaching 2026 tons in 2021, while methamphetamine seizures have stabilized at a high level in 2021 in traditional markets, and expanded elsewhere. On the other hand, illegal manufacture of synthetic drugs is increasing in low- and middle-income countries, including countries with strong regulation. A growing number of laboratories have been detected in Central Asia, Southeast Asia, the Near and Middle East, Africa, Europe, and North America, producing a range of stimulants, depressants, and other novel dissociatives.

Analysis of seized drugs generally implies a two-step process in which a presumptive test is used to provide an indication of the presence or absence of drug classes in the test sample that should be confirmed in the laboratory by high performance analytical instrumentation [2]. Presumptive tests try to maximize the probability of a true results, minimizing, at the same time, the probability of a false negative. Moreover, these tests should be inexpensive nor need instrumentation, and permit on-site measurements by unskilled handlers [3]. A variety of presumptive methods have been developed, including colorimetric tests, microscopy, thin layer chromatography (TLC), and infrared spectroscopy (IR), but many of these tests require instrumentation and/or skilled handlers. Colorimetric tests exhibit appropriate sensitivity, reaching the microgram range [4]. However, those tests present a reduced selectivity, which could result in false positives attributable to the presence of a different drug, adulterants, diluents, or contaminants [5], and are incapable of simultaneously determining a wide variety of compounds. Thus, a cheap, simple, fast on-site test capable of performing multiplex sample analysis is still needed.

Microfluidic paper-based analytical devices (μPADs) with colorimetric detection are a potential solution to this problem [6]. These devices involve chemical or enzymatic tests, which can be

segregated and multiplexed by placing them within wax channels printed on chromatographic paper [7]. With an array of fabrication techniques currently available, paper sensors can now be constructed inexpensively, allowing for multiple applications related to drug analysis in a simple and inexpensive format. Wax patterning was used to create fluidic channels in μPADs [8] to detect different types of psychotropic compounds colorimetrically. The sample solution moved in an area delineated by hydrophobic barriers toward the immobilized reagent via capillary action. The chemical reactions produced visible results that can be interpreted in a few minutes, even with the naked eye. A similar approach using a μPAD for drug identification has been named as idPAD. It was designed to run multiple chemical color tests on illicit drugs, heroin, cocaine HCl, crack cocaine, and methamphetamine, at the same time [9]. The idPAD comprised 12 different color tests designed to detect specific functional groups commonly found in opioids, amphetamines, and other substances of abuse. The test generated a color barcode that can be used to classify the samples by comparison with an image library. Other μPAD devices used gold nanoparticles in combination with specifically tailored aptamers to identify illicit drugs, including cocaine, codeine, and methamphetamine [10]. However, interpretation of a color change is subjective and can be biased by the analyst's perception of color or the environmental illumination conditions [11]. In this sense, smartphone-based devices have received much attention to avoid this subjectivity of the analyst [12]. Smartphone-based analytical devices are simple, easy to use. Nonetheless, these present some potential drawbacks that should be considered. The main ones are related to the lighting environment, the smartphone imaging configuration, and the experimental setup (i.e., relative position between the camera and the sample, stability of the illuminant). To address these issues, mechanical support, such as camera holders and 3D-printed image boxes, have been developed to provide reproducibility in terms of relative position between the target and smartphone camera, and to avoid the influence of environmental light [13].

Most of the traditional colorimetric methods for methylendioxyamphetamine and amphetamine type stimulants recommended by UNODC imply the use of concentrated sulfuric acid, which is not compatible with the use of μPADs. In this sense, microfluidic systems have evolved from the traditional

fabrication of devices using materials such as glass, silicon, and poly(dimethylsiloxane) (PDMS) to 3D printed devices made of polylactic acid (PLA), acrylonitrile butadiene styrene (ABS) or poly(methylmethacrylate) (PMMA). 3D printing has found a niche in (bio)analytical sciences for fabricating devices and sensing platforms at low cost in a high variety of materials [14].

In summary, the aim of the present paper is the development and validation of a 3D-printed smartphone-based device for the classification of illicit drugs, including cocaine, amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA), and synthetic cathinone derivatives using colorimetric reactions in a chemically stable 3D printed microwell platform.

2. Experimental

2.1. Reagents, solutions and samples

Sulfuric acid, hydrochloric acid, and gallic acid were purchased from Merck (Darmstadt, Germany). Sodium nitroprusside, sodium carbonate, and cobalt thiocyanate were acquired from Scharlab (Barcelona, Spain). Formaldehyde solution (37 % w/w), acetone, methanol, and other organic solvents were also obtained from Scharlab.

Milli-Q water (resistivity > 18 MΩ cm, Millipore, Bedford, MA, USA) was used to prepare all solutions. The solution for the gallic acid test was prepared with 0.5 g of gallic acid dissolved in 100 mL of concentrated sulfuric acid [15]. Simon's test with acetone involves two reagents: A and B. Reagent A is prepared by dissolving 1 g sodium nitroprusside in 100 mL of 5 % (v/v) aqueous acetone. Reagent B is prepared with 2 g of sodium carbonate in 100 mL of water. Marquis reagent is prepared by mixing 7 mL of formaldehyde with 100 mL of concentrated sulfuric acid [16]. Scott's test implies two reagents: Reagent A is a 16 % (v/v) aqueous hydrochloric acid solution, and reagent B is a solution of 1 g of cobalt (II) thiocyanate dissolved in 100 mL of water.

A total of 191 seized samples were analyzed, including MDMA, amphetamine and derivatives, cathinone derivatives, cocaine, 3,4-methylenedioxy cathinone derivatives, and pyrrolidine derivatives. Samples were divided into two sets: a first calibration group with 127 samples and a second prediction

group with 64 samples. All these samples of substances were kindly provided for analysis by the Pharmaceutical Inspection and Drug Control Unit of the Health Service Area of the Spanish Government in Valencia (Valencia, Spain).

2.2. Development of the 3D-printed microwell device

Microwell devices were designed using the freeware FreeCAD software (www.freecadweb.org). The device is rectangular (25 x 55 mm) with 5 mm height and includes 15 microwells, divided into three groups of 5. Microwells are designed to be cylindrical with a diameter of 4.0 mm, a depth of 1.0 mm, and an approximate volume of 10 µL.

For positioning and slicing, the model was loaded in computer-aided manufacturing software (Preform, Formlabs, Somerville, USA). The final disposition of the design was transferred to the SLA Form 3 printer (Formlabs) for printing using the FLGPCL04 (Formlabs) clear resin.

After the printing process, the microwell devices were removed from the moving platform and cleaned by bath sonication using isopropanol and deionized water (see Figure 1A and 1B). Then, the objects were dried under a nitrogen stream and finally exposed to UV for one hour (2 × 16 W low-pressure mercury lamps) in a UV oven (KA-9180, PSKY, China). The overall result can be seen in Figure 1A, while an schematic representation is seen in Figure 1B.

2.3. Colorimetric tests for drug identification

Colorimetric tests were performed following the UNODC proposed procedures [https://www.unodc.org/documents/scientific/Screening_Colour_Test_ATS.pdf and https://www.unodc.org/pdf/publications/st-nar-13-rev1.pdf]: approximately, 1 mg of sample is placed in each microwell of a row (5 microwells, one for each reaction), using the tip of a stainless-steel spatula. After that, 5 µL of each test solution was added to the corresponding microwell using a micropipette to perform the colorimetric reactions (sulfuric acid, gallic acid, Simon, Marquis, and Scott tests). If the colorimetric tests need the addition of two reagents, the order is reagent A (5 µL) and

reagent B (5 µL).

After a minimum reaction time of 1 min, the microwell device is introduced in the 3D-printed lightbox for image acquisition.

2.4. Fabrication of the 3D-printed lightbox

Digital images of the microwell device were acquired in a 3D-printed photographic lightbox (16 × 23 x 14 cm). The 3D prototype was designed using FreeCAD software (www.freecadweb.org). Next, the design was printed on a 3D-printer SLA Form 3 printer (Formlabs, Barcelona, Spain). The box was constructed with red PLA filament using the fused deposition filament technique. The top of the box was printed separately to facilitate the introduction of the microwell device. The upper face has a rectangular hole (4 × 2.5 cm) compatible with the dimensions of any smartphone camera for image acquisition.

At the bottom of the lightbox an illumination source box (15 x 21 x 4.2 cm) is introduced (see Figure 1C), which includes five white light emitting diodes (LEDs). The top of the illumination source box consists of a white methacrylate diffuser plate (0.8 cm thick) to guarantee a homogeneous light diffusion and to avoid light saturation during image acquisition. A black board covers the methacrylate diffuser plate with a rectangular hole (5.5 × 2.5 cm) compatible with the dimensions of the microwell device. The LED plates are powered with an external source such as a portable computer, a tablet, a mobile phone, or a rechargeable lithium battery through a USB port or using four AA 1.5 V batteries for system portability. LEDs were positioned in the middle of the bottom face to illuminate the microwell device from the bottom. Additionally, a 2 × 2 cm hole was placed in the lateral face of the lightbox to allow LED plates connection.

The weight of the final prototype (see Figure 1D and E), including the microwell device, lightbox, illumination source box LEDs, and electrical connections, is around 600 g, which enhances portability for *in situ* preliminary identification of psychoactive substances. The computer-aided design (CAD) of the 3D printed device can be found in Supporting Information (Supplementary Figure 1). A smartphone

OSCAL C70 was used for image acquisition.

2.5. Image data treatment and chemometric analysis

All photos were taken using the same experimental and illumination conditions. Images were opened in the free app ColorPicker version 5.9 (https://crea-that.fr) to obtain the RGB values of the colorimetric reaction microwells, adjusting the region of interest as a circular shape to the dimensions of the microwells. RGB values of the reactions were corrected by subtraction of the RGB values of the blanks (microwell device) using the following equation:

$$RGB_{corrected} = 255 - \|RGB_{microwell} - RGB_{blank\ device}\| \qquad \text{(Equation 1)}$$

Where $RGB_{microwell}$ are the original RGB values obtained from the colorimetric reaction and $RGB_{blank\ device}$ are the RGB values obtained from the blank device in the same image. Chemometric models were created from the $RGB_{corrected}$ values for the five colorimetric reactions performed on each analyzed sample.

Chemometric analysis were developed using Solo, version 9.2.1 (Eigenvector Research, Inc., Wenatchee, WA, USA https://eigenvector.com/software/solo)). Different pre-processing methods were evaluated to obtain the selected prediction models.

In this sense, classification models were developed using 67% of the samples (127) for calibration and 33% for validation (64), including samples from all the different types (see Table 1). Sample selection was carried out by using the Kennard-Stone algorithm [17]. Autoscale of data was applied before algorithms were applied.

Several chemometric models were developed using Partial Least Squares Discriminant Analysis (PLS-DA), a linear classification method based on the PLS regression algorithm [18], and Artificial Neural Networks (ANN) [19].

The constructed models were internally validated by the venetian blinds (20%) cross-validation method. Model parameters such as the R^2 coefficient, the root mean squared error of calibration, and the root mean squared error of cross-validation (RMSEC and RMSECV) were considered for selecting

the best model. The prediction capacity of the models was evaluated with different statistical parameters, such as the root mean squared error of prediction (RMSEP) of the validation set samples.

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{n} (y_i - \hat{y}_i)^2}{n-1}} \qquad \text{(Equation 2)}$$

Where y_i is the reference value for the validation set sample i, $\hat{y}_i$ is the predicted value for validation set sample i, and n is the number of samples in the validation set. The plot of the explained variance against the number of factors (latent variables) enabled the determination of the optimum number of factors in the case of PLS-DA. Then, the initial model was refined by selecting those factors, resulting in the lowest RMSEP for the validation set and closest values to 1 for sensitivity and specificity coefficients.

Sensitivity and specificity were calculated to evaluate classification and authentication models, being sensitivity the ability of the model to correctly classify positive samples as positive.

$$Sensitivity = \frac{(TP)}{(TP+FN)} \qquad \text{(Equation 3)}$$

where: TP = number of positive samples classified as positive; FN = number of positive samples classified as negative. Specificity is the ability of the model to correctly classify negative samples as negative.

$$Specificity = \frac{(TN)}{(TN+FP)} \qquad \text{(Equation 4)}$$

Where: TN = number of negative samples classified as negative; FP = number of negative samples classified as positive.

2.6. Reference methodologies for drug identification

Samples were accurately identified in the laboratory using reference procedures based on gas chromatography with mass spectrometry detection (GC-MS), infrared spectroscopy (IR) using the attenuated total reflectance (ATR) sampling mode, nuclear magnetic resonance (NMR) [Drug Test. Analysis 2015, 7, 280–289] and direct infusion high resolution mass spectrometry (HRMS) [Journal of Chromatography A, 1574 (2018) 91–100]. Details of the experimental procedures can be found in the

supplementary material section.

3. Results and discussion

3.1. Development of microwell devices

The material employed for 3D-printing of the microwell device should have a series of properties such as chemical stability. First of all, it should be considered that most of the colorimetric reactions employed for illicit drug identification include aggressive chemicals, like strong acids (e.g., sulfuric acid). Thus, the material used in the microwell device should be stable under such aggressive conditions. Moreover, given that the analyzed sample is a solution, direct lighting from the upper part of the 3D device (like, for example, the smartphone's flash or any other illumination system that implies a light source on top of the sample) would potentially create reflections which will make automatic post-processing of the image very difficult. Under these restrictions, the best alternative is to illuminate the sample from the bottom, so that no interferences arise. In this sense, the presence of a diffusive material between the light source and the liquid is desired to ensure that all the microwells were homogeneously illuminated, an aspect that was considered in the preparation of the setup (section 2.4.). Considering all these aspects, the FLGPCL04, a methacrylate-based clear resin, has been selected, as it is not affected by the addition of the aforementioned reagents during short periods of time.

On the other hand, the geometries of the wells have been designed to reduce the consumption of samples (1.0 mg per reaction) and reagents (5 μL per well) and, at the same time, to avoid shadow formation for image acquisition.

3.2. Operational parameters and optimization of the lightbox

Digital images can be obtained using a smartphone in two different configurations: open and closed systems. The open system only uses the smartphone and therefore, factors such as ambient light, sample position and distance affect the analytical features of the colorimetric analysis. On the other hand, all the aforementioned factors can be controlled using a photographic light box as a closed

system for image acquisition. To compare both systems, the microwell device was filled with three replicates of a MDMA sample, and the colorimetric tests were performed following the aforementioned procedures. Supplementary Figure 2 shows the image acquired using the open configuration versus the image acquired using the closed system. As can be seen, in the open configuration, the reflection of ambient light and shadows could be an important aspect affecting the repeatability of the measurements. However, in the closed system configuration, the sample is illuminated from the bottom through a white methacrylate diffuser plate to guarantee a homogeneous diffusion of light, avoiding shadows and reflections.

Moreover, it should be also considered that smartphone camera features such as filters, lenses and lens aperture, will also affect image acquisition. Thus, the effect of using different smartphones for image acquisition was also investigated. To do that, the microwell device was filled with three replicates of a methylone sample, and the colorimetric tests were performed following the aforesaid procedures. In this case, the images of the microwell device were obtained using different smartphones, including an OSCAL C70, a Samsung S8+, an Oppo Reno 2Z, a Xiaomi 11 Lite 5g, and an Apple iPhone 11. Supplementary Table 1 shows the average colors obtained for each colorimetric reaction (three replicates) and each evaluated smartphone. As can be seen, the color was drastically affected by the smartphone used in image acquisition. For instance, the Marquis reaction provided a yellow-green color using the OSCAL smartphone or a dirty yellow when the Samsung and Xiaomi were used. Thus, a subtraction of the background was proposed using equation 1, in which the difference between the RGB values of the colorimetric reaction was subtracted from those of the background, and the absolute value of this difference was subtracted to 255. As it can be seen, after background subtraction, the effect of the different optical and color filters used in digital cameras is substantially minimized. Thus, the corrected RGB values would be used for chemometric data treatment.

3.3. Colorimetric reactions of representative compounds

Figure 2 shows the color obtained from the reactions with the representative compounds of the

different families evaluated. which included cocaine samples, MDMA, amphetamine, cathinone and derivatives, pyrrolidine cathinone derivatives, and 3,4-methylenedioxy cathinone.

Amphetamine and methamphetamine form a reddish-, and a yellowish-orange-colored product, respectively, with Marquis reagent due to the formation of the carbenium ion [16]. It has been reported that some other amphetamine and cathinone derivatives also yield yellow, yellowish green, and other colors. As has been said, it is possible to differentiate between amphetamine derivatives that are substituted at the aromatic part and those that are not substituted. The reaction with concentrated sulfuric acid only produced colored products with substituted derivatives (MDMA, MDPV, and methylone), although the structures and the reaction mechanisms have not yet been clarified.

On the other hand, secondary amines, such as MDMA, methamphetamine, and methylone can be identified with Simon reagent by forming a blue-grey complex. The amine and acetaldehyde produce the enamine, which subsequently reacts with sodium nitroprusside, and the immonium salt is hydrolyzed to the Simon-Awe complex. Additionally, it has been observed that the pyrrolidine derivatives provide a greenish complex, clearly identifiable, after the addition of Simon reagents.

The Scott Test has been developed to differentiate between cocaine and other compounds using the cobalt(II)-thiocyanate reagent. As can be seen in Figure 2, cocaine samples provided an intense blue color after the reaction with the Scott reagent. However, false positive results could be obtained for pyrrolidine derivatives, which also provide the intense blue color complex. Recently, a study proposed the application of the partial least squares discriminant analysis (PLS-DA) method associated with digital images obtained by a smartphone camera to increase the selectivity of the Scott test. In this work, the spectral behavior and color development of cocaine and false positive substances was also assessed [Forensic Science International Volume 335, June 2022, 111277]. Moreover, acidification of the solution with hydrochloric acid leads to a solubilization of many blue precipitates obtained from adulterants, but not for the cocaine precipitate [K. Oguri, S. Wada, S. Eto, H. Yamada, Specificity and mechanism of the color reaction of cocaine with cobaltous thiocyanate, Jpn. J. Toxicol. Environ. Health, 41 (1995), pp. 274-279]. An extraction of the drug precipitate by adding chloroform, which results in a

two-phase solution, allows to separate the aqueous phase pink-colored and the organic one turned blue.".

Limits of detection (LOD) were estimated for cocaine and MDMA samples. To do that, solid samples were prepared by grinding a known weight of the illicit substance (cocaine and MDMA) and the cutting agent (lactose) together in a mortar. This mixture was serially diluted by adding lactose, and was analyzed by the recommended procedure. Finally, the limit of detection was established, considering the minimum amount of drug (concentration in the sample in % w/w) that allows a correct classification using the established procedure. LODs were 40 and 80 µg, corresponding to 2 and 4 % w/w, for cocaine and MDMA., respectively, which are similar to those previously reported for this type of devices [8,9].

3.4. Chemometric analysis

First of all, principal component analysis (PCA), an unsupervised algorithm, was used to evaluate the classification capability of the set of corrected RGB values. Given that a total of 191 samples were analyzed, and that each sample was subjected to 5 different reactions (each one yielding 3 channels, RGB), a final matrix of dimensions [191x15] was obtained, representing 2865 data points. The first three principal components in the PCA model captured 85% of the total variance. Figure 3 shows the representation of the samples in the space defined by the PC1-PC2 (Fig. 3A) and PC1-PC2-PC3 (Fig. 3B). As it can be seen, MDMA and 3,4-methylenedioxy cathinone samples can be clearly differentiated from the rest of evaluated drugs. However, cocaine, pyrrolidone amphetamine, and cathinone derivatives form a big cluster with an unclear differentiation in the unsupervised model.

The PLS-DA supervised classification model allows the separation of classes using predictive (correlated) components. Based on the lowest value of the RMSECV, six latent variables were chosen to build the classification model. After selecting the number of latent variables, the performance of the classification model was evaluated by internal validation (cross-validation) and external validation (prediction set). It should be highlighted that a minimum strict threshold of 0.50 has been selected as

a classification rule. It proved to have a good R^2 for calibration from 0.65 to 0.95, cross-validation from 0.59 to 0.95, and prediction from 0.66 to 0.99.

Moreover, Table 2 shows the root mean square error of calibration (RMSEC), cross-validation (RMSECV), and prediction (RMSEP) and the classification error, also for calibration, cross-validation, and prediction, for the evaluated classes. The classification performance of the model was evaluated through the confusion matrix obtained on cross-validation and sample prediction (see Table 3). From these data, sensitivity, and specificity were calculated.

The architecture of the ANN was first optimized for the data, which were divided into the training and test sets by the Kennard-Stone algorithm. The input values were autoscaled variable by variable, and the backpropagation leaming rule was used, being, once again, the goal of net training to minimize the RMSECV. Moreover, to avoid overfitting, the performance of the network is tested with an independent prediction set. The ANN utilized in this study consisted of two active layers of nodes (6 nodes in layer 1 and 2 nodes in layer 2) with a sigmoidal transfer function. Once again, a minimum strict threshold of 0.50 has been selected as the general classification rule. It clearly improved the PLS-DA results, with R^2 values for calibration from 0.96 to 0.99, cross-validation from 0.89 to 0.99, and prediction from 0.79 to 0.99. Table 2 shows the data obtained for the classification error and the RMSE for calibration, cross-validation, and prediction for the different classes obtained using the ANN model. The classification performance of the model was evaluated through the confusion matrix obtained on cross-validation and sample prediction (see Table 4). From these data, sensitivity and specificity were also calculated.

The results obtained with this study had been compared with those previously published. For instance, cocaine has been previously identified using the digital images acquired after the Scott test and chemometric evaluation via Support Vector Machine Discriminant Analysis (SVM-DA) [Microchemical Journal, Volume 127, July 2016, Pages 87-93] and PLS-DA [Forensic Science International, Volume 335, June 2022, 111277]. SVM-DA and PLS-DA correctly classified all samples, yielding sensitivity and specificity equal to 1. Additionally, digital image analysis of colored tests has demonstrated a

338 significant potential for the development of an accurate, rapid, portable, and economically viable semi-
339 quantitative analysis of amphetamine and methamphetamine in seized samples [Drug Testing and
340 Analysis, Volume3, Issue5, May 2011, Pages 277-282]. Another approach was based on the image
341 acquisition of twelve colorimetric tests to differentiate illicit drugs, distractor substances, and cutting
342 agents [Journal of Forensic Sciences, Volume65, Issue4, July 2020, Pages 1289-1297]. The device
343 showed 95% sensitivity and 100% specificity for detecting these drugs. In summary, the results
344 obtained in this study demonstrated that the developed device, based on PLS-DA and ANN, could be
345 considered acceptable and performed well in the classification of illicit drug samples.
346
347 **3.5. Study of interferences**
348 The developed sensor has been evaluated from a selectivity point of view. Thus, potential
349 interferences of some of the major known adulterants, mixing agents, and other psychoactive
350 substances from different families have been obtained and analyzed. In this study, a total of 28
351 different compounds, including benzocaine, mannitol, starch, tetracaine, levamisole, lidocaine,
352 cellulose, paracetamol, acetylsalicylic acid, ketoprofen, caffeine, phenacetin, ascorbic acid, piracetam,
353 procaine, phenylephrine, tramadol, mescaline, methallylescaline, benzofuran compounds (6-EAPB and
354 6-APB) and 2C-X family compounds (2C-E, 2C-B, 2C-P, DOC, 2C-B and 2C-B fly) have been analyzed.
355 The results show that benzofuran compounds provided a dark violet color in the Marquis reaction and
356 a pink to orange color in the gallic and sulfuric acid reactions. Moreover, levamisole and tetracaine
357 provided a blue color in the Scott test, and 2C-X family, phenylephrine, and tramadol gave an orange-
358 yellow color in the Marquis reaction. The rest of the analyzed compounds did not react with the
359 assayed reagents or provided uncolored products.
360 Interferent compounds have been analyzed following the developed PLS-DA and ANN methodologies.
361 In these cases, PLS-DA classifies two samples as cocaine, two samples as MDMA, 17 samples as
362 amphetamine and cathinone derivatives, and seven samples as unassigned class, with the percentage
363 of correctly classified samples of only 25 %. On the other hand, ANN classifies two samples as cocaine,

two samples as MDMA, and 24 samples as unassigned class, with the percentage of correctly classified samples of 86 %. Samples classified as cocaine by both models corresponded to levamisole and tetracaine, which provided a blue color in the Scott test. Samples classified as MDMA by both procedures corresponded to benzofuran analogs, which provided dark color products in the Marquis, gallic, and sulfuric acid reactions. Finally, while PLS-DA failed to classify most adulterants and mixing agents, ANN correctly classified them in an unassigned class.

4. Conclusions

A 3D-printed analytical device has been successfully developed for classifying illicit drugs using smartphone-based colorimetry. Representative compounds of the different families, including cocaine, MDMA, amphetamine and cathinone derivatives, pyrrolidine cathinones, and 3,4-methylenedioxy cathinones, were analyzed and classified after appropriate reaction with Marquis, gallic acid, sulfuric acid, Simon and Scott reagents. A picture of the colored products was acquired using a smartphone, and the corrected RGB values were used in the chemometric treatment. ANN using two active layers of nodes (6 nodes in layer 1 and 2 nodes in layer 2) with a sigmoidal transfer function and a minimum strict threshold of 0.50 identified illicit drug samples with a sensitivity higher than 83.4 % and a specificity of 100% with limits of detection in the microgram range.

The 3D printed device can operate connected to a rechargeable lithium-ion cell portable battery, is inexpensive, and requires minimal training. The analytical device has been able to discriminate the analyzed psychoactive substances from cutting and mixing agents, being a useful tool for law enforcement agents to use as a screening method.

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392

Author contributions:

J.C. and S.A. conceptualization, methodology and design, formal analysis and investigation, writing-original draft, funding acquisition and resources, supervision.

M.L.C. and A.E.M.R. writing-review and editing, funding acquisition and resources.

R.S.H. writing-review and editing.

Notes: The authors declare that they have no financial/commercial conflicts of interest. Furthermore, it should be highlighted that under any circumstances, the authors haven't trafficked, proportionated, facilitated, or forced any volunteer to consume illegal substances.

Legend of Figures:

Figure 1. 3D-printed microwell device picture (A) and schematic representation (B); illumination source box (C); 3D-printed analytical device (D) and digital image obtained for a methylone sample (E).

Figure 2. Colored products developed for representative examples of the selected families analyzed in this study.

Figure 3. Unsupervised PCA PC1 vs. PC2 (A), PC1 vs. PC2 vs. PC3 (B) obtained for RGB data on analyzed samples.

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