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TECHNICAL NOTE

CRIMINALISTICS

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Validation of Powder X-Ray Diffraction Following EN ISO⁄IEC 17025

ABSTRACT: Powder X-ray diffraction (PXRD) is used widely in forensic science laboratories with the main focus of qualitative phase identification. Little is found in literature referring to the topic of validation of PXRD in the field of forensic sciences. According to EN ISO⁄IEC 17025, the method has to be tested for several parameters. Trueness, specificity, and selectivity of PXRD were tested using certified reference materials or a combination thereof. All three tested parameters showed the secure performance of the method. Sample preparation errors were simulated to evaluate the robustness of the method. These errors were either easily detected by the operator or nonsignificant for phase identification. In case of the detection limit, a statistical evaluation of the signal-to-noise ratio showed that a peak criterion of three sigma is inadequate and recommendations for a more realistic peak criterion are given. Finally, the results of an international proficiency test showed the secure performance of PXRD.

KEYWORDS: forensic science, powder X-ray diffraction, validation, EN ISO⁄IEC 17025, laboratory proficiency test, trueness, specificity, selectivity, robustness, preparation errors, detection limit

Little is found in literature referring to the topic of validation and powder X-ray diffraction (PXRD), especially relating to the field of forensic sciences (1). This paper gives a summary of how required parameters can be examined and used for validation report.

Validation according to EN ISO⁄IEC 17025 is "(…) the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled" (2, p. 41). Point 5.4.5.3 in EN ISO⁄IEC 17025 states that "the range and accuracy of the values obtainable from validated methods (…) as assessed for the intended use, shall be relevant to the customer's needs" (2, p. 41). The main intended use of PXRD in forensic science laboratories is qualitative phase identification (3,4). To meet the expected standards, the PXRD method is tested for parameters of trueness, specificity, selectivity, robustness against preparation errors, and detection limit.

Materials and Methods

Powder X-Ray Diffraction

The device used for PXRD analysis is the D8 Advance (Bruker AXS GmbH, Karlsruhe, Germany) with transmission, $\theta/2\theta$ geometry, and a position-sensitive Våntec detector. The sample rotates with constant angular speed throughout the measurement. Different apertures are available with a diameter of 3, 6, and 15 mm. The following measurement parameters are used if not stated otherwise (Table 1).

Powders are prepared between two Kapton® foils (8 µm; Spex-Certiprep®, Stanmore, U.K.) on a specimen holder (Fig. 1).

The specimen holder is inserted in a sample holder which is placed into a magnetic holder of the D8 Advance. The evaluation software used is Diffrac Plus Release 2005 Eva Version 11.0 from Bruker AXS GmbH. Reference database used is ICDD PDF2 of 2005 and self-established user databases. If not stated otherwise, diffractograms are evaluated by import of raw files in Eva, followed by background subtraction and Fourier transformation.

Micro X-Ray Fluorescence

To support the evaluation of diffractograms, most laboratories apply elemental analysis as well. In this work, elemental analysis is performed by micro-X-ray fluorescence (µ-XRF). The device used is Eagle II (Röntgenanalytik Messtechnik GmbH, Taunusstein, Germany) with an Si(Li)-detector. With the help of mono-capillary optics, small sample quantities can be analyzed. Measurement parameters and evaluation of spectra are carried out with the software Eagle II Vision 32. The sample preparation is the same as with the PXRD, so samples analyzed with both methods do not change preparation between the two measurements. The following parameters are used for elemental analyses if not stated otherwise (Table 2).

Weighing

Two balances were used: Mettler model AE 163 (Mettler Toledo GmbH, Gießen, Germany) and a Cahn-25 balance (Fisher Scientific, Schwerte, Germany).

Sample Materials

Zinc oxide, calcite, sodium chloride, potassium chloride, lithium fluoride, lead carbonate, galactose, lactose, arabinose, xylose, sorbose, glucose, fructose, mannitol, talc, and cellulose were purchased from Merck (Darmstadt, Germany); caffeine and lactose monohydrate from Fluka Chemie GmbH (Buchs, Switzerland); lead sulphate from Riedel de Haën (now Fluka Chemie GmbH); SRM 2686 Portland Cement Clinker from the National Institute of

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TABLE 1—*Measurement parameters for PXRD analysis.*

Radiation	Cu $K_{\alpha 1}$ 1.540598Å
Voltage	40 kV
Current	40 mA
Measurement range	5–80° 2θ
Time/step	0.1 sec
2θ range/step	0.0086°
Overall measurement time	14.55 min
Rotation	30 r/min
Aperture	6 mm

PXRD, powder X-ray diffraction.

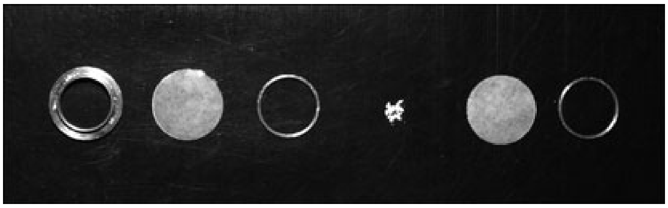

FIG. 1—*Sample preparation of powder X-ray diffraction (PXRD).*

TABLE 2—*Measurement parameters for μ-XRF analysis.*

Anode Material	Rhodium
Voltage	40 kV
Current	Variable (2500 cps)
Atmosphere	Vacuum
Spot size	300 μm
Overall measurement time	300 sec
Type of measurement	10-point analysis

μ-XRF, micro-X-ray fluorescence.

Standards and Technology (Gaithersburg, MD); SRM 674 α-aluminium oxide and titanium oxide from the National Bureau of Standards (Gaithersburg, MD); the soil standard "Light alluvial-deluvial meadow soil" PS-3 COD No 312a-98 from MBH Analytical Ltd. (Barnet, UK); silver material from Ventron GmbH (Karlsruhe, Germany); and gold material from Balzers Union (now Leica Microsystems, Wetzlar, Germany). Commercially available primers (Magtech Cleanrange [Magtech Ammunition Company, Inc., Lino Lakes, MN], Geco-Sinoxid [RUAG Ammotec GmbH, Fürth, Germany], and S&B nontox [Sellier Bellot J.S.C., Vlaim, Czech Republic]) were provided by KT23, BKA, Wiesbaden, Germany. Heroin sample was seized by KT34, BKA. No certificate of analysis was available of the explosives, trinitrotoluene, pentaerythritol tetranitrate, and trinitro-2, 4,6-phenylmethylnitramine, and so they were examined by nuclear magnetic resonance spectroscopy.

Survey and Results

Trueness

The parameter trueness should be tested by analyzing certified reference material or material that is already analyzed with another validated method in case no certificate is given. In qualitative analysis, the only conclusion drawn is the phase identification.

Materials chosen to test this parameter are lactose monohydrate, aluminium oxide, the soil standard PS-3, the Portland cement clinker, trinitrotoluene, pentaerythritol tetranitrate, and trinitro-2,4,6-phenylmethylnitramine.

Identification of a single phase of crystalline material is in general unproblematic. The identification of lactose hydrate and the three explosives was without difficulties.

The problem with discrimination between a single phase and a mixed crystal comes up with the examination of the phase corundum. The evaluation software Eva proposes besides pure Al_2O_3 a mixed crystal $Al_{1.98}Cr_{0.2}O_3$, whose pattern can only be differentiated from pure Al_2O_3 pattern with the support of elemental analysis information (Fig. 2).

A greater complexity is shown by the examination of the cement and soil standards, which consist of multiple crystalline phases (Tables 3 and 4).

The main diffraction peaks of alite (PDF no. 055-0740) and belite (PDF no. 033-0302) overlay each other (Fig. 3). The only free diffraction peaks of belite are the ones around $44.1° < 2\theta < 44.8°$ or *c.* 37.3°. The lower intensity of the belite phase (Ca_2SiO_4) complicates the identification, and furthermore, the presence of a solid solution, for example, $(Ca_{1.99}Mg_{0.01})SiO_4$, cannot be excluded. Periclase, which in total shows only five diffraction peaks in the range of $5° < 2\theta < 80°$, can only be identified with the support of elemental analysis and the knowledge that it may be a typical cement phase.

As can be seen in Fig. 4, even with a measurement time of 4 h, chlorite, which holds nine weight percent, cannot be identified. Referring to illite/muscovite, only a hint of presence of a mica group mineral can be given. The matrix absorption, crystallinity, and stray radiation prevent the identification of crystalline phases with lower weight percentage. An operator can see diffraction peaks (e.g., $2\theta = 33.1°$ or $2\theta = 39.2°$) that are still left over, but even with the support of elemental analysis no secure identification of other crystalline phases is possible.

Specificity and Selectivity

If a qualitative analysis result is to be given with a certain method, the parameters' specificity and selectivity have to be tested. The criterion of specificity is achieved if the relevant analyte can be identified or quantified. The criterion of selectivity is fulfilled if all analytes can be identified separately. Both parameters can be tested with a synthetically generated phase composite of certified reference materials. Referring to PXRD, the success of identifying one or more phases in a phase mixture is strongly depending on the quality of phase crystallinity, the presence of amorphous compounds, and similar crystal structures (e.g., multiple mica phases, multiple cubic metal phases, or mixed crystal systems). If a phase mixture consists of multiple compounds whose diffraction peaks overlay each other, secure identification can be problematic.

The ability of PXRD to differentiate between crystallographic similar patterns or to reach this ability with the support of elemental analysis is tested. Other compound mixtures consist of multiple phases and are analyzed for example to test how many phases can be detected in one mixture.

Although the following sample mixture is not normally analyzed by the method of PXRD in real casework scenario, it is a good example to show the method's limitations. The analysis of a mixture of gold and silver with PXRD is problematic. Multiple phases are suggested by the search/match routine of the Eva software (e.g., OV, LiH, Pd_3Gd, and CuBr), all consisting of different elements (Fig. 5).

All phases belong to the crystallographic space group Pm3m or Fm3m, and it is impossible to say which pattern could be used for the identification because all of them match the diffractogram. On the contrary, with the help of elemental analysis, it is clear that the mixture consists of silver and gold.

A mixture of seven sugars (consisting of galactose, lactose hydrate, arabinose, xylose, sorbose, glucose, and fructose to 1/7 weight percent each) is analyzed. With a measurement time of 1 h

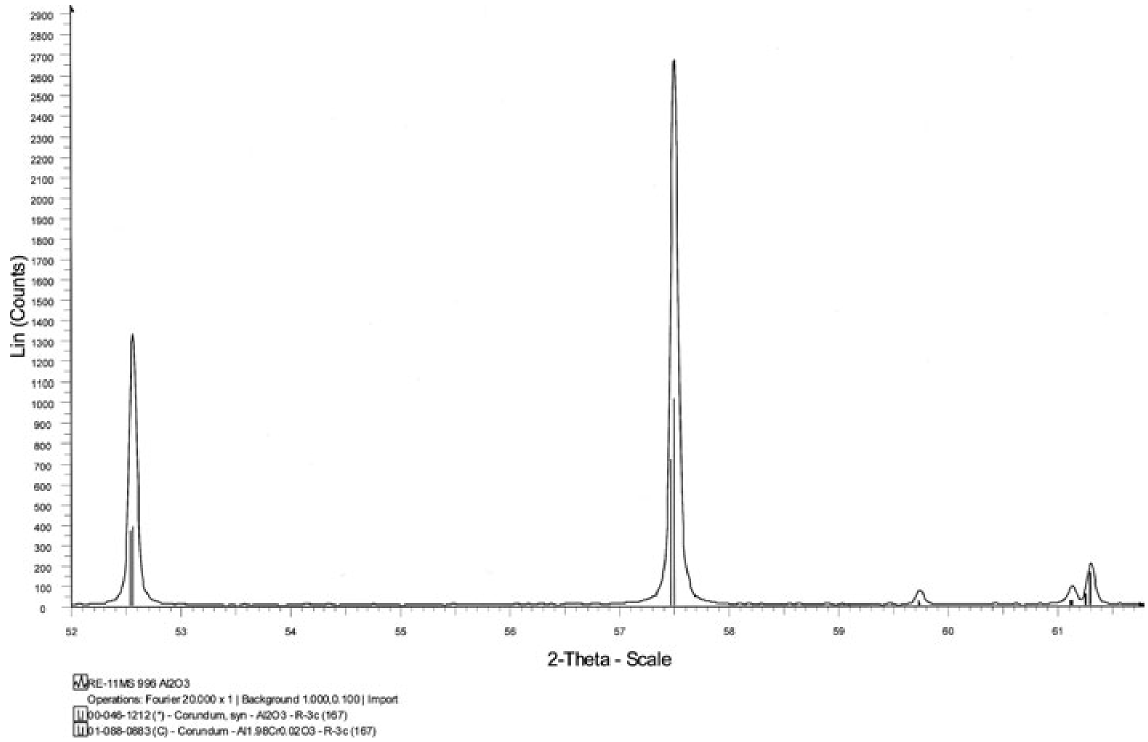

FIG. 2—*Diffractogram of the sample* Al_2O_3.

TABLE 3—*Phase composition of SRM 2686 Portland cement clinker as given by certificate.*

Phase	Mass Percent
Alite (C_3S)	58.6 ± 4.0
Belite (C_2S)	23.3 ± 2.8
Ferrite (C_4AF)	14.1 ± 1.4
Periclase (MgO)	3.3 ± 1.9
Aluminate (C_3A)	2.3 ± 2.1

C, CaO; S, SiO_2; A, Al_2O_3; F, Fe_2O_3.

TABLE 4—*Phase composition of soil standard PS-3 as given by certificate.*

Phase	Weight Percent
Quartz	28
Albite	18
Orthoclase	14
Chlorite	9
Calcite	8
Illite + Muscovite	7
Tremolite	6
Montmorillonite	2
Goethite	2
Gibbsite	2
Rhodochrosite	1
Organic compounds	1

and the support of elemental analysis, the seven sugar phases could be clearly identified (Fig. 6).

A likewise mixture consisting of 10 phases (zinc oxide, titanium oxide, aluminium oxide, silicon oxide, calcium oxide, lead carbonate, lead sulphate, sodium chloride, potassium chloride, and lithium fluoride) was prepared as an example of an inorganic mixture. All phases could be identified with a measurement time of 1 h and the support of elemental analysis (Fig. 7).

Robustness Against Preparation Errors

A disadvantage of the focusing geometry of the powder diffractometer is the high sensitivity for errors in sample height. These errors can appear through an improper fixation of the clamping rings or the preparation of the powder between the two Kapton® foils. To evaluate the consequences of such errors, test samples were consciously prepared falsely.

Inhomogenic Particle Size Distribution

Primer composition samples show in general a heterogeneous material with phases consisting of different particle size. Because of the sensitivity to friction and compression, grinding of such substances can be dangerous and has to be carried out with great care. Caused by the different particle size and the upright position of the sample during a measurement, there is a possibility that only bigger particles are fixed by the strain of the two Kapton® foils. As a consequence, material loss could occur with smaller particles falling out of the irradiated area and the corresponding phases would not be detected.

To evaluate the consequences of such a problem, three primers were prepared in three different ways. The comparison of the three S&B nontox samples is shown for example (Fig. 8). The first way of preparation is as stated in the general sample preparation technique (Fig. 8*A*), the second preparation includes a grinding step (Fig. 8*B*), and the third one uses the grinding step plus a powder fixation on the lower Kapton® foil with the help of silicon oil (Fig. 8*C*). The silicon oil should improve the adhesion of particles to the foil and prevent particle loss.

No material loss or missing phases could be observed comparing the three different preparation techniques.

The Magtech samples showed a displacement of $\Delta 2\theta = 0.02°$. During the grinding step, ungrindable spheres were observed, and some were sorted out for further measurements. Scanning electron

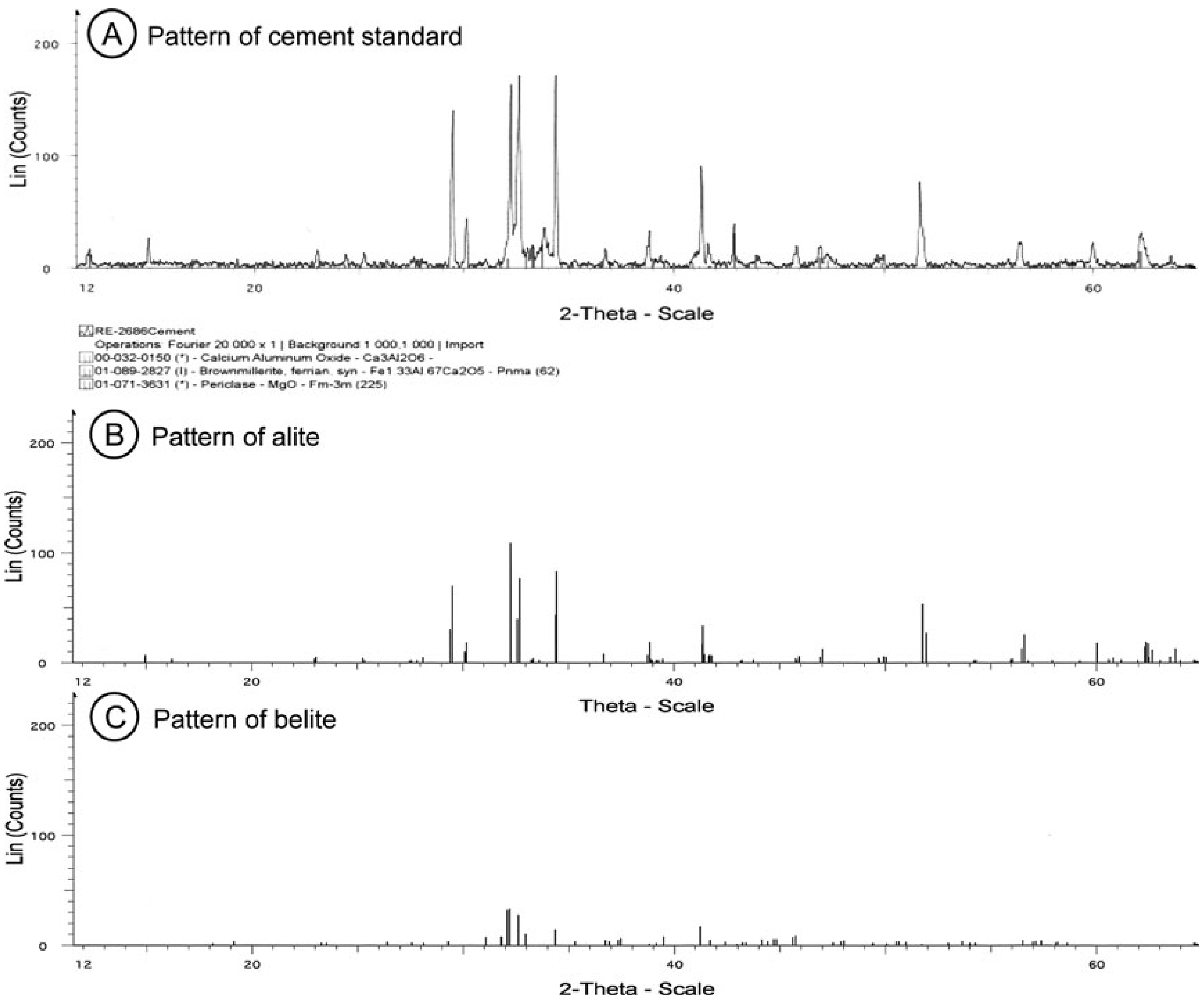

FIG. 3—*Diffractogram of SRM 2686 Portland cement clinker.*

microscopy of the amorphous particles showed only oxygen, nitrogen, and carbon. It is concluded that these particles consist of nitrocellulose that is a typical composite of primers.

Texture Effects

Preparation of layered minerals, for example phyllo-silicates, can be difficult. Because these minerals show a layered crystallographic structure and are also elastic, grinding of such materials is complicated. When the material is fixed between the two Kapton® foils, a preferential orientation parallel to the foil occurs. This effect can be enhanced by the statical interaction between, for example, micas and the Kapton® foils. In consequence, an adequate number of statistically oriented crystals are not warranted. Selected diffraction peaks are given a much higher intensity, whereas others will not be detected caused by their positioning in the measurement geometry. This causes a problem for the evaluation software, because the resulting pattern does not match the ones in the reference database. Pulverizing a mica mineral (Fig. 9*A*) for 2 (Fig. 9*B*), 8 (Fig. 9*C*), and 20 min (Fig. 9*D*) lowers the texture effect.

The best match with muscovite is given after pulverizing for 20 min because more diffraction peaks are detected and can be assigned to the pattern of the reference database.

Variations in Sample Geometry

Consequences of variations in sample geometry need to be evaluated. It is common that different operators prepare a different mass of material between the two Kapton® foils or even one operator if preparing the same material multiple times. Another error can occur in case one clamping ring is not properly fixed (Fig. 10).

The specimen could also be inserted upside down into the magnetic holder of the D8 Advance. A sample could be prepared too thick or too thin. Consequences of these errors were tested on an inorganic sample (the cement standard) and an organic sample (heroin).

It is shown that a thinner (Fig. 11*C*) than normal preparation (Fig. 11*A*) of organic material results in a strong intensity loss, whereas a thicker preparation (Fig. 11*B*) shows a strong intensity gain.

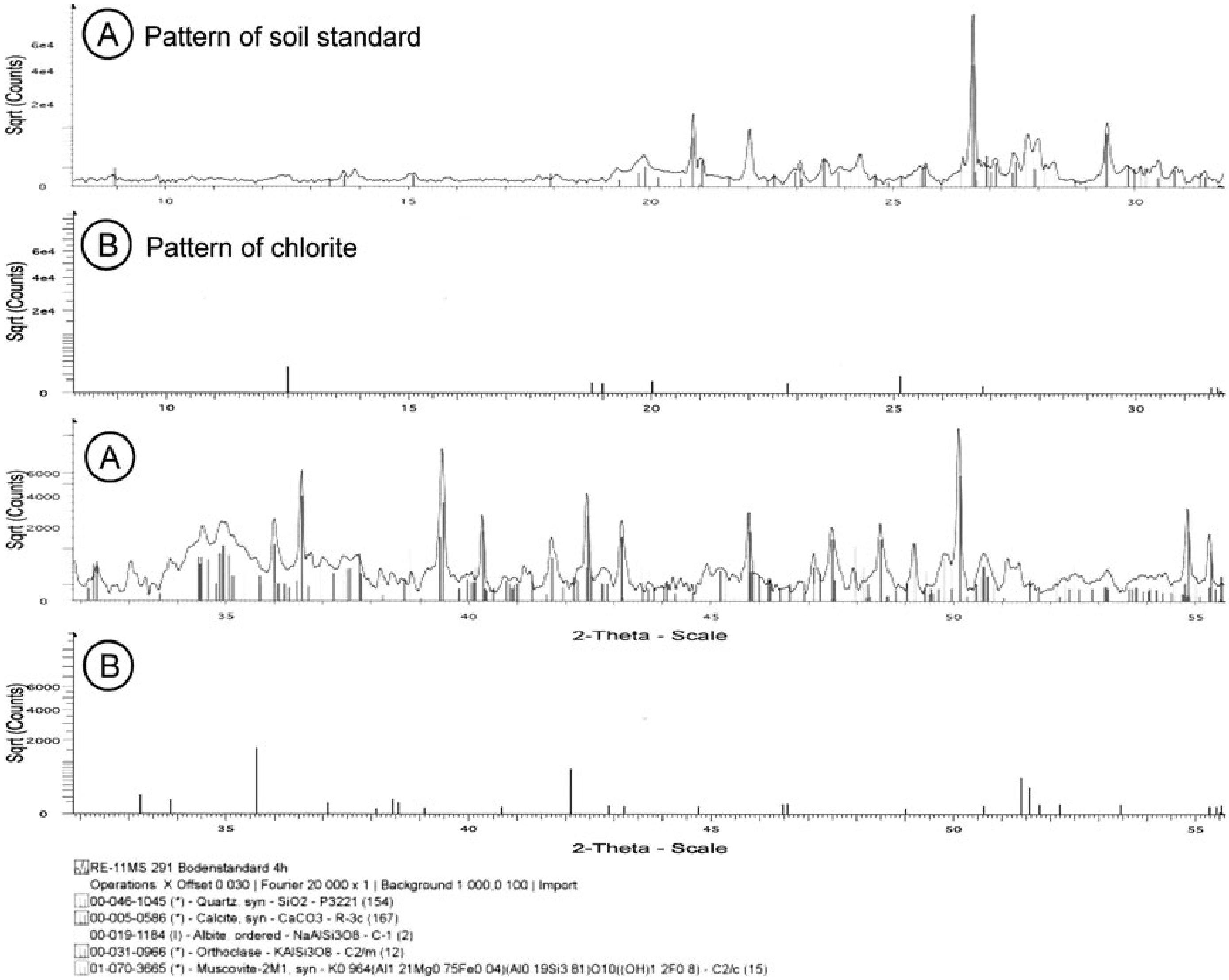

FIG. 4—*Diffractogram of the soil standard PS-3.*

Because inorganic materials have higher absorption coefficients, a thicker (Fig. 12*B*) than normal preparation (Fig. 12*A*) results in an extremely intensity loss, whereas the thinner preparation (Fig. 12*C*) does not show a great effect on diffracted intensity.

A displacement of the pattern for about $\Delta 2\theta = 0.003°$ when the clamping ring is not properly fixed (Figs 11*D* and 12*D*) occurs because of the aberration of the focusing geometry. This effect can be observed more clearly when the specimen holder is inserted into the magnetic holder upside down (Figs 11*E* and 12*E*). The difference in height from the focusing plane to the sample surface plane is about 3.5 mm and results in a displacement of about $\Delta 2\theta = 0.6°$. The evaluation software gives the possibility to enlarge the accepted displacement of diffraction peaks for the search/match routine. Although with a displacement of $\Delta 2\theta = 0.6°$, there is actually no chance for the software to suggest any proper phases.

Operator-Dependent Errors

Operator-dependent preparation errors were investigated by multiple measurements of a certified reference material. First of all, the soil standard was prepared once and measured 10 times directly one after another without taking the sample holder out of the D8 Advance or change of sample preparation. This should give an approximate range of measurement uncertainty which can then be compared to the intensity variations caused by operators.

Three different operators prepared the soil standard 10 times. One operator has a preparation experience for 30 years, the second one has no preparation experience, and the third one has about a 5-year preparation experience. To compare the diffractograms on a statistical basis, the criterion I_{netto}, full width at half-maximum, and chord middle are determined and evaluated with the Eva software for selected diffraction peaks in the raw data. I_{netto} is the difference between the absolute measured intensity of a diffraction peak and the intensity of the background at that 2Theta position. Three diffraction peaks of the quartz phase ([101], [100], and [122]), two diffraction peaks of the calcite phase ([104] and [116]), and one diffraction peak of the albite [−201] were integrated with Eva software, followed by the determination of the arithmetic mean of the net intensities including span widths.

It can be seen in Fig. 13 that the statistical device measurement uncertainty is relatively low compared to the one introduced by operators and multiple preparation times.

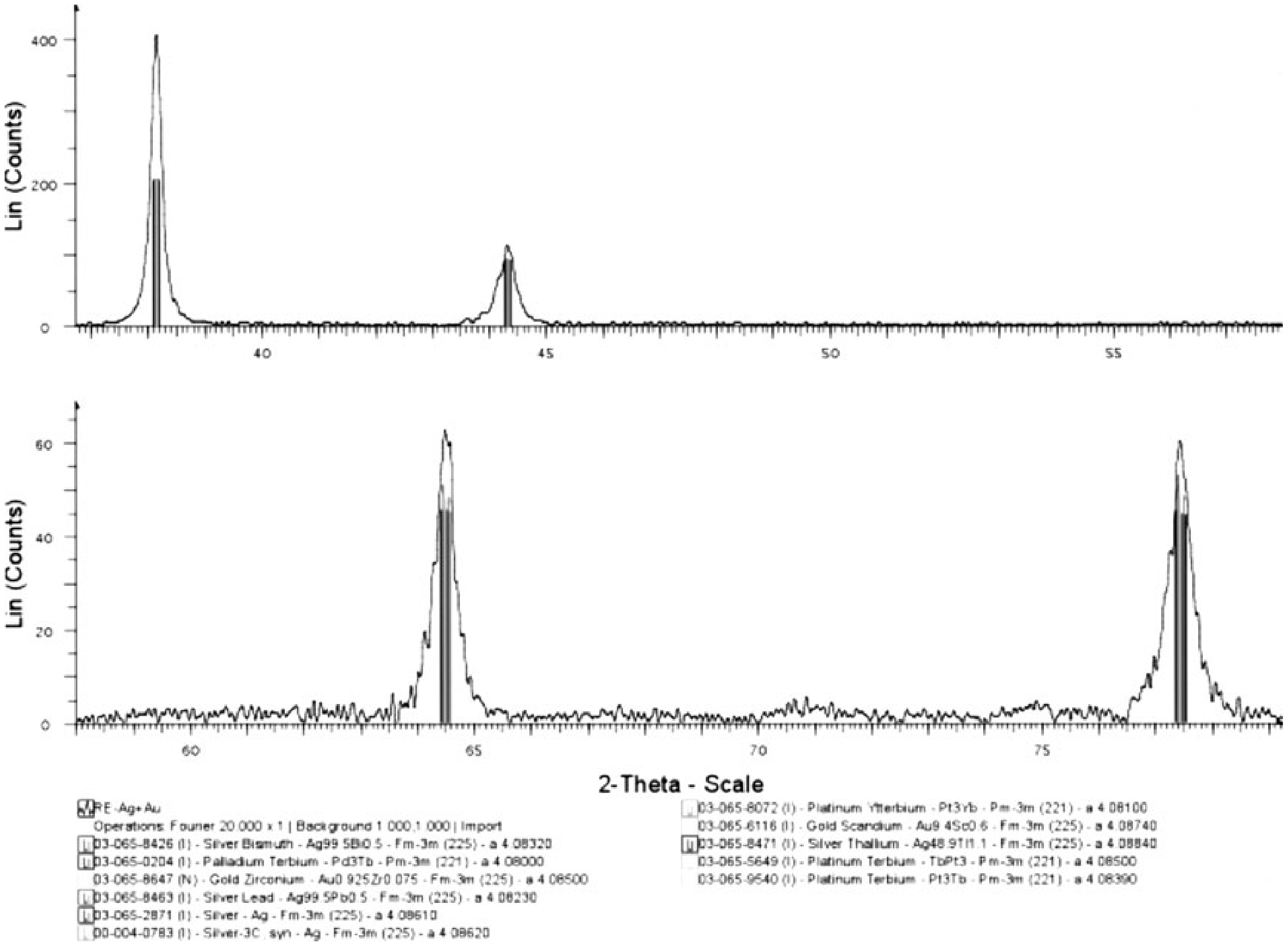

FIG. 5—*Diffractogram of Ag/Au mixture.*

The relative errors of operator A are the smallest ones, so in this example a 30-year experience in preparation results in a more reproducible intensity distribution compared to the operators with lower or no preparation experience. Nevertheless, although the highest standard deviation of intensity was found to be 30%, no difference in phase identification was given by three different operators.

Detection Limit

The reproducibility of diffracted intensities is in qualitative phase analysis important in case diffraction peaks of phases with low weight percentage are identified in one measurement and not identified in a repeated measurement or new preparation because of intensity variation. For the examination of detection limits, it was differentiated between the detection limit of an absolute sample quantity minimum of a pure phase and a detection limit of a certain phase in a phase mixture.

The detection limit is typically determined as the lowest quantity of a substance that can be distinguished from the absence of that substance or from the background noise. According to He (5), the standard deviation is given by

$$\sigma_B = \sqrt{N_B}$$

where N_B is the background counts.

Applied to PXRD, the criterion for the presence of a diffraction peak is therefore given as:

$$N_{\text{brutto}} - N_B \geq x \times \sqrt{N_B}$$

with N_{brutto} being the total counts in the diffraction peak, and x being a factor of confidence. Choosing $x = 3$, for example, means that the counts above this limit have a probability of 99.87% of truly belonging to a diffraction peak and not to background noise.

It is tested whether the above formula is applicable to PXRD and whether a criterion of $x = 3$ is adequate. Measurement tests are carried out for samples of varying density and a fluorescent sample as well to see whether there are any implications for the peak criterion. Test material includes Kapton® foils, silicon oil, MgO, ZnO, ZnS, PbS, and Fe_2O_3 which were prepared between two Kapton® foils and measurement with all available apertures. In case of background measurement of Kapton® foils, 4 Kapton® foils were prepared between the two Kapton® foils used for fixation. The identical sample was used for the measurement with the three apertures.

After measurement, the diffractograms are imported into Eva software, followed by a background subtraction (curvature 1, threshold 1). The resulting files are transformed to an x–y file with the program Powder-Cell for Windows Version 2.4 (Bundesanstalt

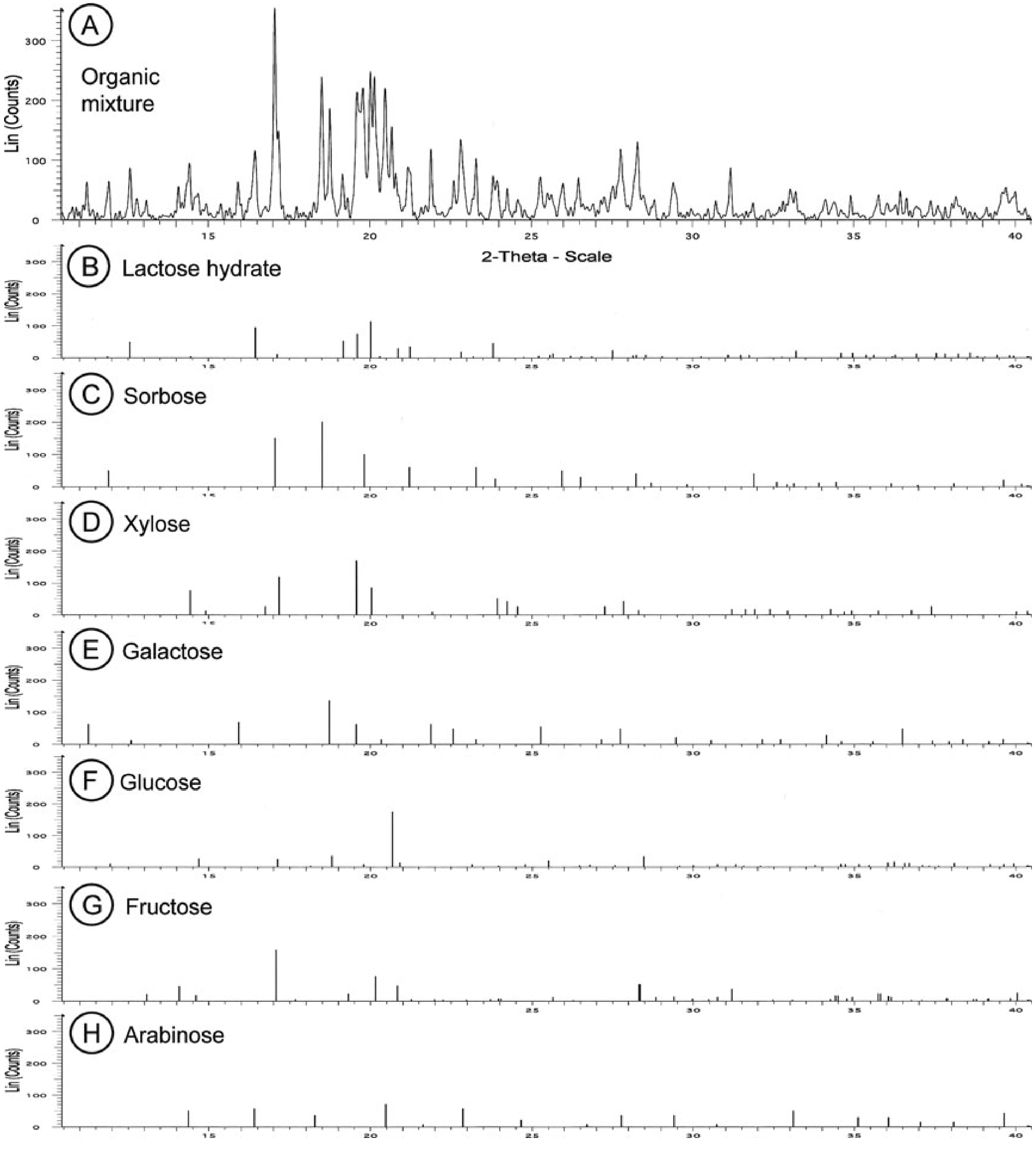

FIG. 6—*Diffractogram of the organic mixture.*

für Materialforschung und -prüfung, Berlin, Germany) (W. Kraus and G. Nolze, programmers), so the data can be read by Excel. Three ranges of $2\theta = 5°$ (581–582 single measured intensities) without any diffraction peaks or contribution from amorphous compounds were selected in each diffractogram in the range between $10° \leq 2\theta \leq 70°$. In the case of α-Fe_2O_3, only 650 single measurements could be selected in the range of $60° \leq 2\theta \leq 70°$ for background examination. The determination of the factor x was carried out in Excel by calculating the arithmetic mean of measured intensities within the 5° range, its standard deviation, and the difference in the arithmetic mean (I_{MW}) to the maximum (I_{max}) and minimum (I_{min}) single measurement. It was then calculated how often the standard deviation is contained in the maximum difference to retrieve the factor x.

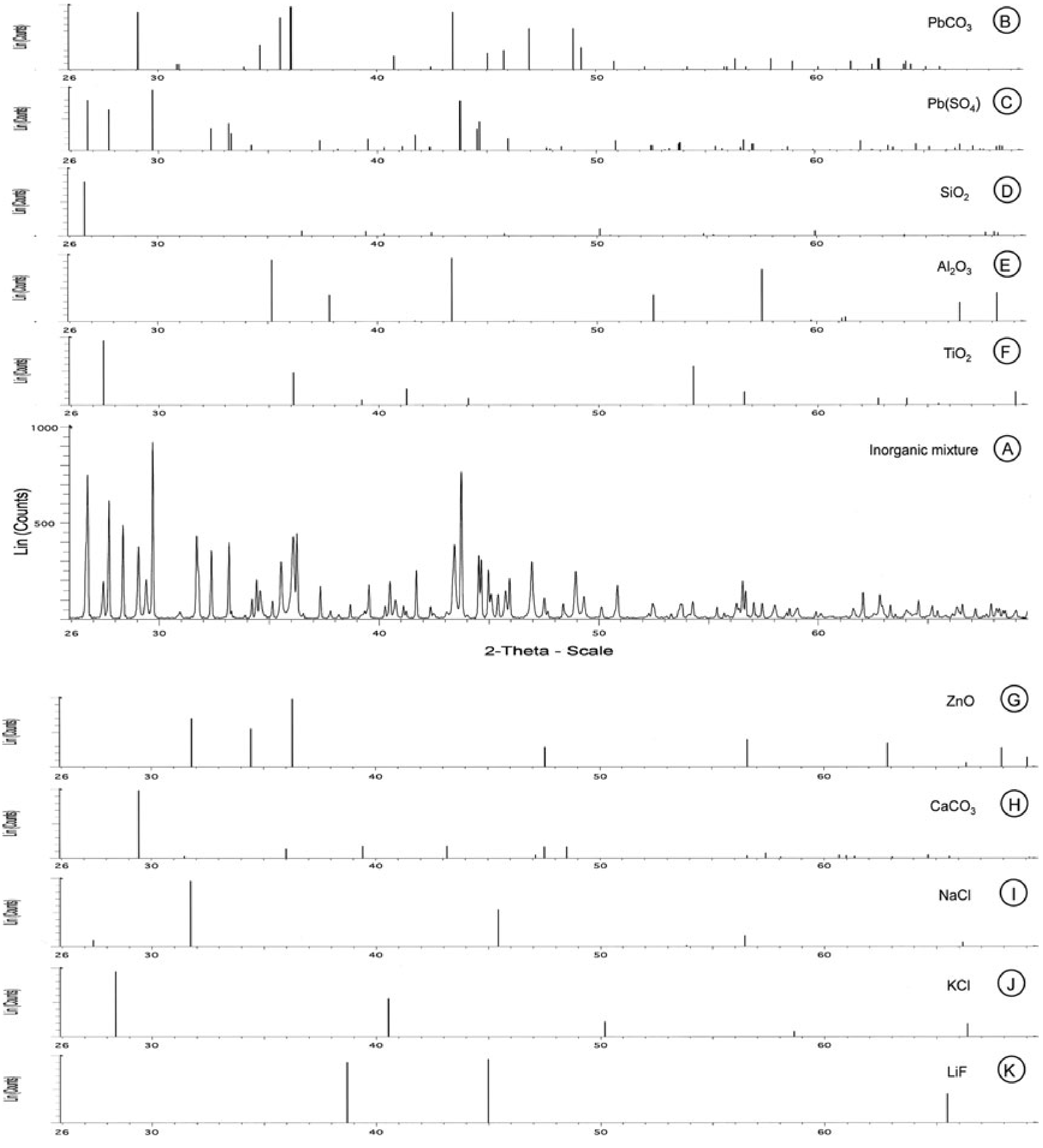

FIG. 7—*Diffractogram of inorganic mixture.*

$$x = \frac{I_{\max} - I_{\mathrm{MW}}}{\sigma} \qquad x = \frac{I_{\mathrm{MW}} - I_{\min}}{\sigma}$$

An example of the background examination of the 4 Kapton® foils measured with the 6-mm aperture is shown (Fig. 14).

For any measured material, there are nine calculations of the kind shown above (3 apertures × 3 5° ranges). For calculation of the factor x, the particular highest value is selected. Table 5 shows a summary of the results of the particular highest value for the particular aperture used and the corresponding 2θ range.

The calculations show that a factor of $x = 3$ is not high enough to adequately include the statistical scattering of background noise. The calculated factors of 3.98 and 3.95 indicate that even a factor of 4 could be too low in some cases. It should be paid

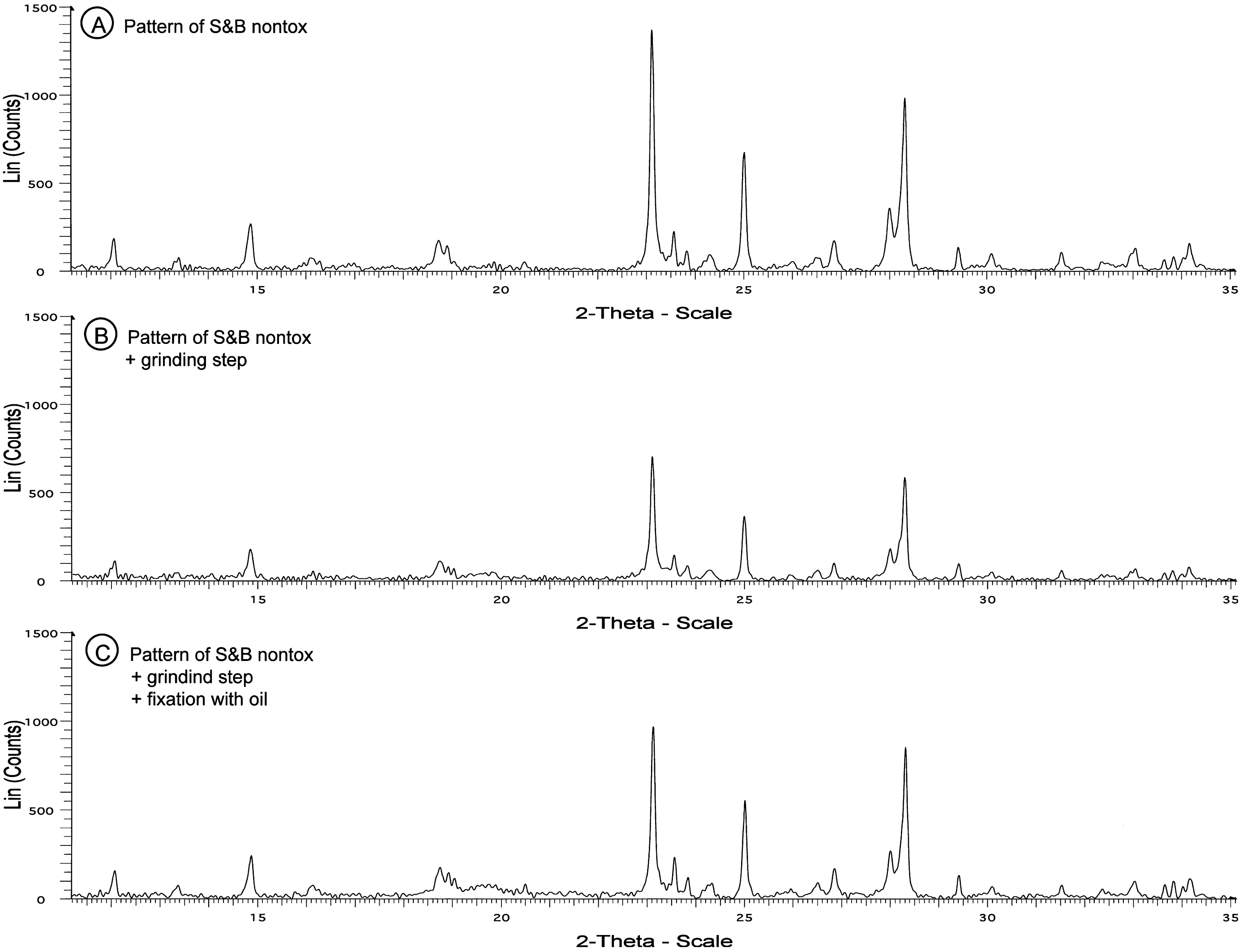

FIG. 8—*Diffractograms of the three differently prepared S&B nontox samples.*

attention to the fact that a background calculation to identify a diffraction peak later on is not carried out over such a large 2θ range. Because the examination in this work should lead to a fundamental conclusion, a large 2θ range was selected for statistical analysis.

Pitfalls in Mathematical Smoothing of Raw Data

When evaluating a diffraction pattern, diffractograms are in general prone to a background subtraction (curvature 1, threshold 1) and a Fourier transformation (factor 20 × 1). The background subtraction does not change the ratio of the measured intensities at all. Although a Fourier transformation, which is a way to facilitate phase identification, is not an ideal tool for the examination of diffraction peaks near the limit of detection, the sine terms in the mathematical formula of the Fourier transformation automatically create a peak form. The arrows in Fig. 15 indicate where the mathematical transformation automatically creates a peak form above the five-sigma criterion without the presence of diffraction peaks.

The Fourier-transformed diffraction pattern (Fig. 15*A*) shows a clear periodicity traceable through the wave function. Independent of the smooth function the operator used, he may question the marked "peaks" which are even above a five-sigma criterion (an arithmetical mean of 50 counts leads to a five-sigma criterion of 85.4 counts). If transforming the diffractogram by the function "smooth," the degree of periodicity fades (Fig. 15*B*). Although the marked intensities are not differentiable from background noise in the raw data, a mathematical transformation generates an unfavorable situation for phase identification. To overcome such a problem, the original raw data should always be imported for the evaluation of possible diffraction peaks near the limit of detection. The presence of a true diffraction peak is most probably given if the "peaks" can be seen in the raw data as well and if multiple following measurement values lie above a (in this case) five-sigma criterion.

It should be kept in mind that the total diffraction pattern is examined. The presence of one single diffraction peak usually cannot be used for the secure identification of a phase. In addition to overcome such a problematic situation, the detection limit can be doubled when measurement time is increased by a factor of four. If there is a chance to identify more phases with longer measurement time, it should always be preferred if enough time is given.

For the calculations of detections limits, a diffraction peak criterion of $N_{\text{brutto}} - N_B \geq 5 \times \sqrt{N_B}$ is used.

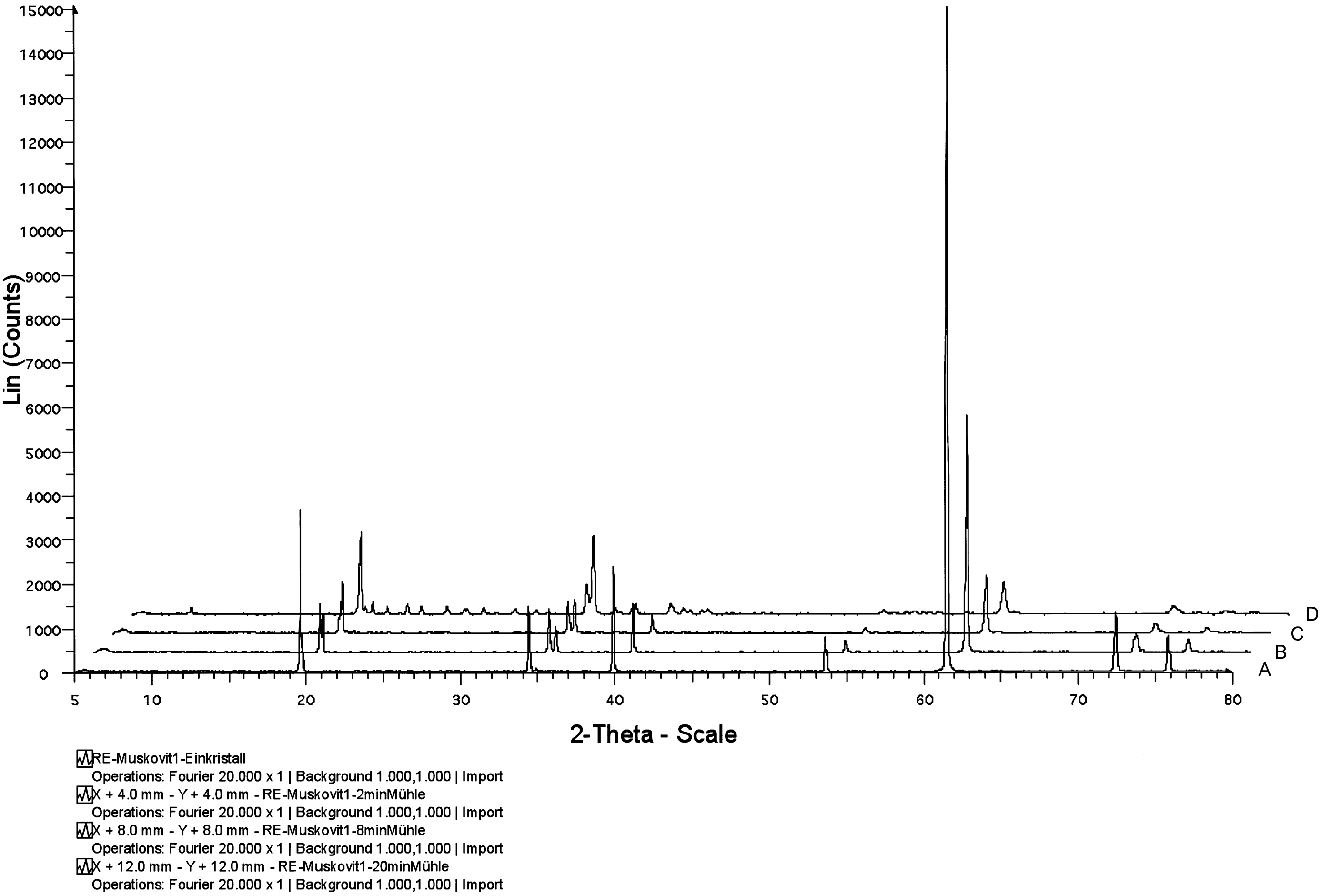

FIG. 9—*Diffractogram of muscovite pulverized for 0, 2, 8 and 20 min.*

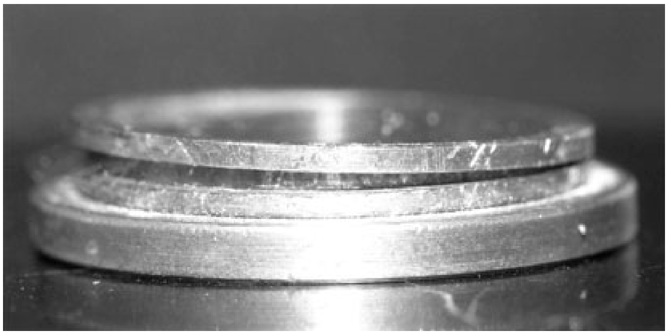

FIG. 10—*Improper fixation of the upper clamping ring.*

Detection Limit of an Absolute Sample Quantity Minimum

The detection limit of a pure single crystalline phase is affected by "background noise," scattering power, and absorption coefficients of the phase irradiated. The determination of the detection limit of a pure phase is tested with lactose hydrate, quartz, and lead oxide. The materials were weighed directly onto the Kapton® foil that is already fixed to the metal specimen holder (the Cahn-25 balance was used) in the range from 15 to 60 μg for each phase. After the weighing step, the sample was taken out of the balance, and the second foil was directly fixed to the sample. The samples were measured with the 15-mm aperture. Diffractograms were background-subtracted (curvature 1, threshold 1) and Fourier-transformed (factor 20 × 1). The tool "area" of the Eva software calculates information such as net intensities of selected diffraction peaks, which were used for the examination of the peak criterion. A substance is clearly identified if more than four diffraction peaks fulfill the criterion $N_{\text{brutto}} - N_{\text{B}} \geq 5 \times \sqrt{N_{\text{B}}}$.

The detection limits for single-phase crystalline components as lactose monohydrate, quartz, and lead oxide are found to be 61.6, 28.1, and 15.4 μg, respectively. This is easily explained as scattering power rises owing to electronic density of the irradiated material. As samples were analyzed for only 15 min and the aperture of 15 mm was used, it is probable that detection limits are lowered by longer measurement time and application of a smaller aperture. Although the data give a good estimation of detection limits of pure phases measured with the D8 Advance, the effect of crystallinity, crystal size, and matrix composition should be obeyed.

Detection Limit of a Certain Phase in a Phase Mixture

Four mixtures of forensic relevance are prepared in the concentration range of interest.

The two organic mixtures contain the agent MDMA-HCl (3, 4-methylenedioxymethylamphetamine hydrochloride) in an ideal matrix. Mixture 1 consists of about 75 weight percent cellulose, 6 weight percent talc, 6 weight percent magnesium stearate, and 12

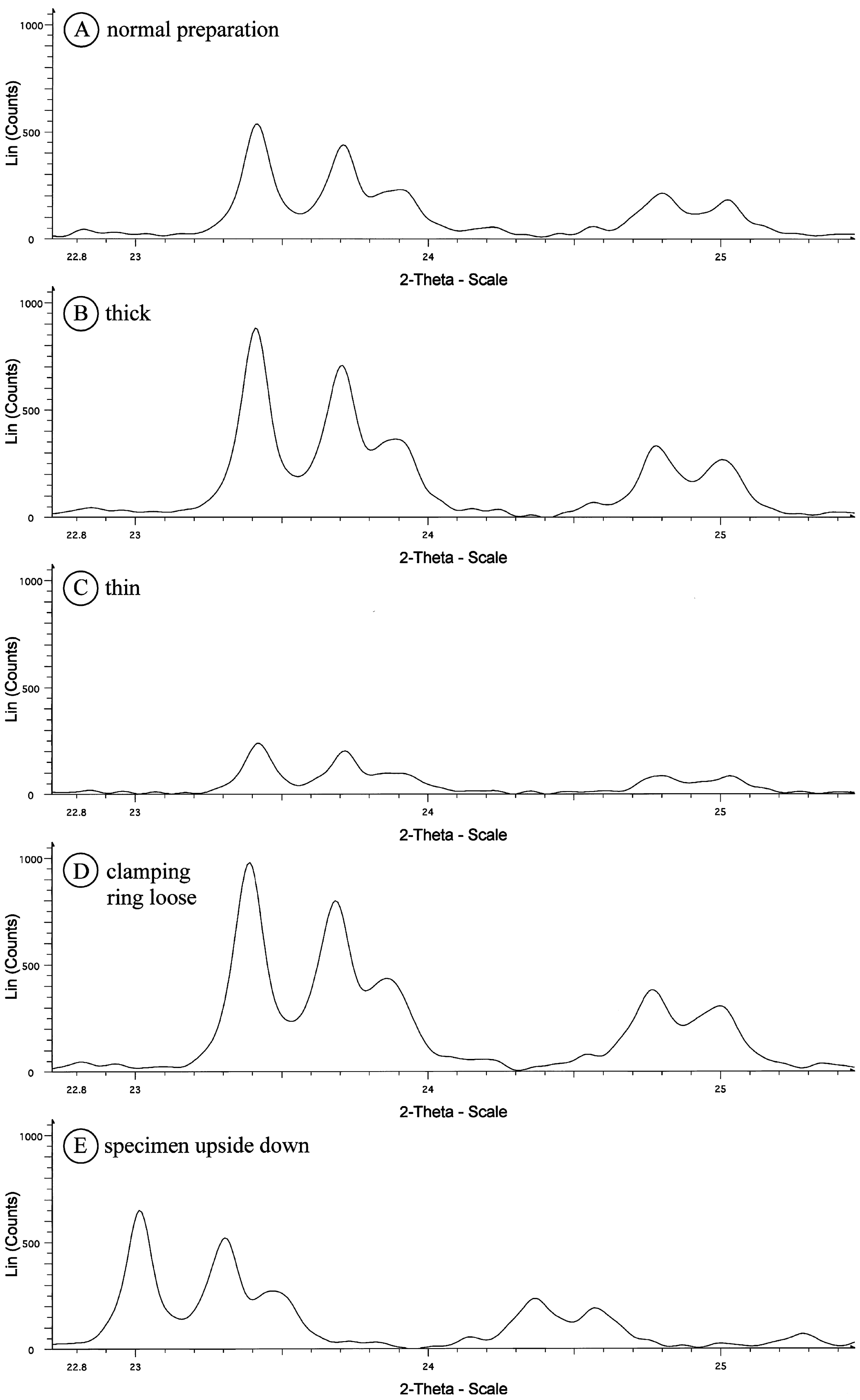

FIG. 11—*Diffractogram of heroine: results of possible preparation errors of an organic sample.*

weight percent caffeine. Matrix 2 consists of about 66 weight percent cellulose, 7 weight percent of talc and magnesium stearate each, and 21 percent of lactose hydrate. Table 6 shows the composition of the matrices.

The composites were weighed with the Mettler balance and homogenized for 15 min in an agate mortar. The agent MDMA-HCl was added to these matrices in different amounts (Table 7). Again, a phase is identified if more than

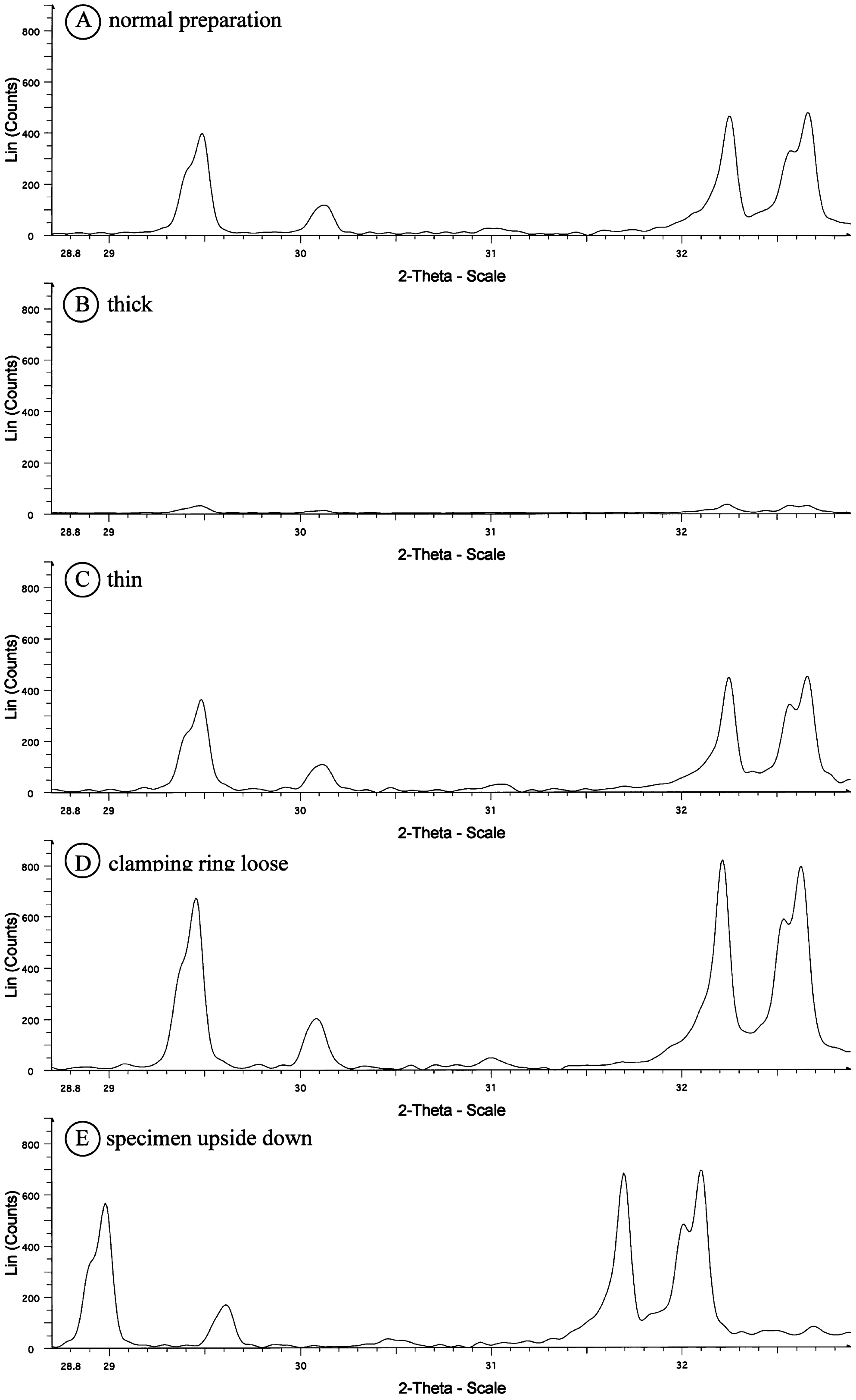

FIG. 12—*Diffractogram of cement standard: results of possible preparation errors of an inorganic sample.*

four diffraction peaks match the pattern of the reference database. Results show that detection limit of MDMA-HCl in matrix one lies around 3 weight percent and in matrix two around 2 weight percent.

The inorganic mixtures were weighed together using the Mettler balance as Table 8 shows and homogenized with a swing mill (Retsch GmbH, Haan, Germany) for 2 min. Detection limit of calcite in quartz is found to be around 2 weight percent, and the detection limit

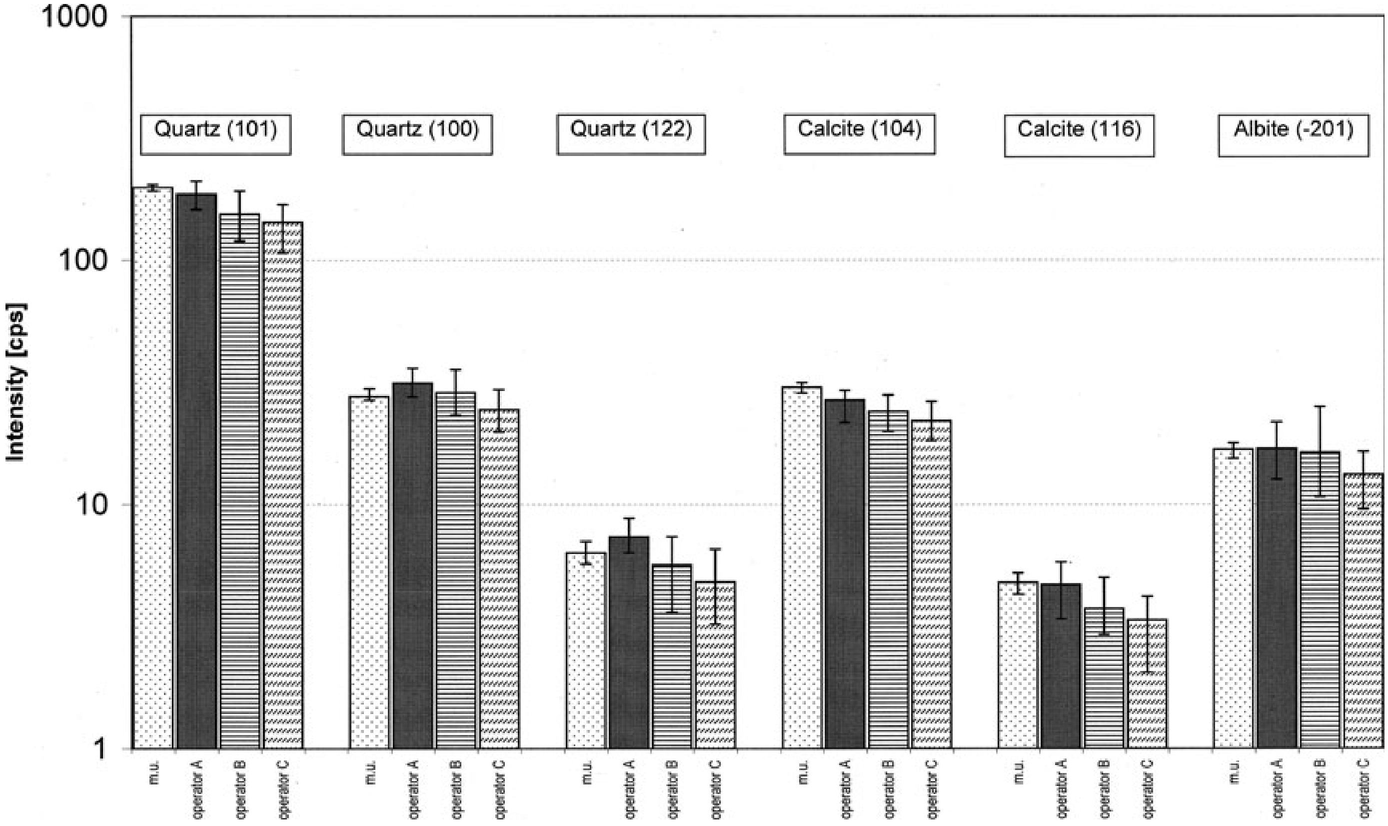

FIG. 13—*Comparison of intensity variation in selected diffraction peaks.*

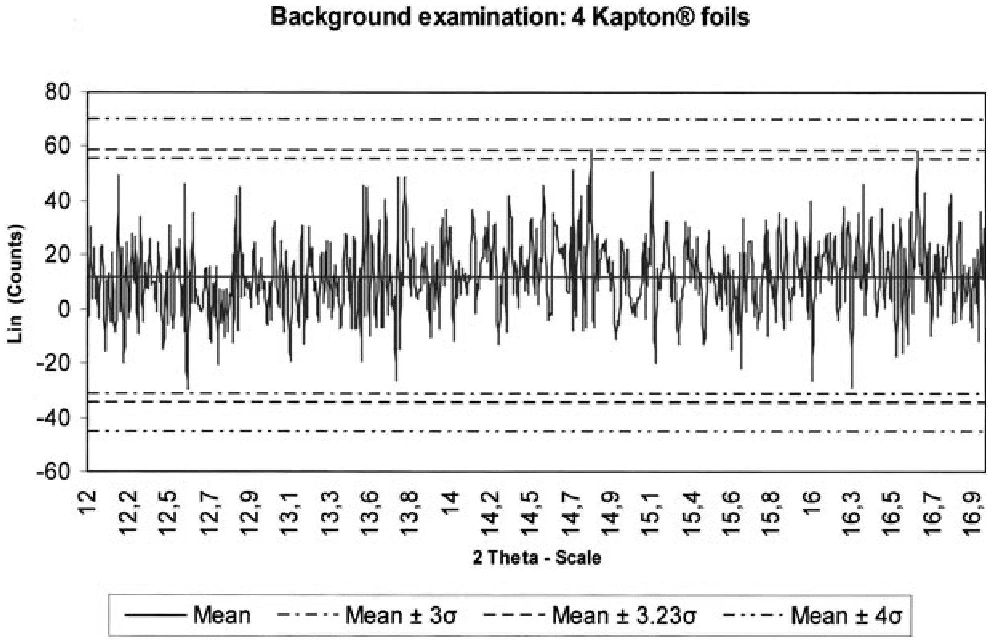

FIG. 14—*Background examination of 4 Kapton® foils measured with 6 mm aperture.*

of quartz in calcite lies around 3 weight percent. This is easily explained as calcite has a higher scattering power than quartz.

As the test results of the MDMA-HCl in matrix 2 resulted in 2 weight percent of MDMA-HCl being detected and 3 weight percent not being detectable, blind test was carried out.

With the help of blind tests, false-positive and false-negative findings can be examined. A false-positive results mean that a phase is identified that is not contained in the sample. A false-negative finding states that a phase that is actually present is not identified. The test was carried out with two operators. Operator A

TABLE 5—*Calculation of the factor* x.

Sample Name	*x*	2-Theta-Range
Scatter 4 Kapton® foil 3-mm aperture	3.56	12–17°
Scatter 4 Kapton® foil 6-mm aperture	3.23	12–17°
Scatter 4 Kapton® foil 15-mm aperture	3.29	26–31°
Scatter silicon oil 3-mm aperture	3.67	10–15°
Scatter silicon oil 6-mm aperture	3.55	30–35°
Scatter silicon oil 15-mm aperture	3.98	50–55°
Scatter MgO 3-mm aperture	3.43	10–15°
Scatter MgO 6-mm aperture	3.70	10–15°
Scatter MgO 15-mm aperture	3.66	10–15°
Scatter ZnS 3-mm aperture	3.95	39–44°
Scatter ZnS 6-mm aperture	3.77	38–43°
Scatter ZnS 15-mm aperture	3.34	50–55°
Scatter ZnO 3-mm aperture	3.20	39–44°
Scatter ZnO 6-mm aperture	3.61	50–55°
Scatter ZnO 15-mm aperture	3.50	10–15°
Scatter PbS 3-mm aperture	3.95	10–15°
Scatter PbS 6-mm aperture	3.33	55–60°
Scatter PbS 15-mm aperture	3.52	36–41°
Scatter Fe_2O_3 3-mm aperture	3.53	42–47°
Scatter Fe_2O_3 6-mm aperture	3.89	10–15°
Scatter Fe_2O_3 15-mm aperture	3.40	42–47°

prepared samples that were preselected from operator B and tested for the presence of the selected compound. A false identification has especially in forensic laboratories fatal juristic consequences.

As Tables 9 and 10 show, no false-positive or false-negative results were reported.

TABLE 6—*Composition of the two tablet matrices.*

	Cellulose (wt%)	Talc (wt%)	Mg-Stearate (wt%)	Caffeine (wt%)	Lactose Hydrate (wt%)
Matrix mixture 1	75.0	*c.* 6.3	*c.* 6.3	12.5	0.0
Matrix mixture 2	*c.* 64.3	*c.* 7.1	*c.* 7.1	0	*c.* 21.4

As the operator examined the diffractogram for a known substance, it is obvious why 0.4 weight percent of calcite can give a hint to the substance, whereas the detection limit lies around 2 weight percent.

It should be kept in mind that a mixture is rarely totally homogeneous, especially if the prepared sample amount lies around only a few tens of milligrams. Every substance or mixture is individual, so there can be no general statement as "PXRD/D8 Advance has a detection limit of *x* weight percent." The tests carried out should only give a general idea for a detection limit for PXRD and the device used.

Laboratory Forensic Proficiency Test

Another method to prove the trueness of the results of a method is to take part at proficiency tests. Because such a procedure is recommended and proficiency tests carried out by scientific community are rare, such a test was organized by the German Federal Criminal Police Office. One operator generated the samples and organized the test, whereas another operator tested the unknown samples.

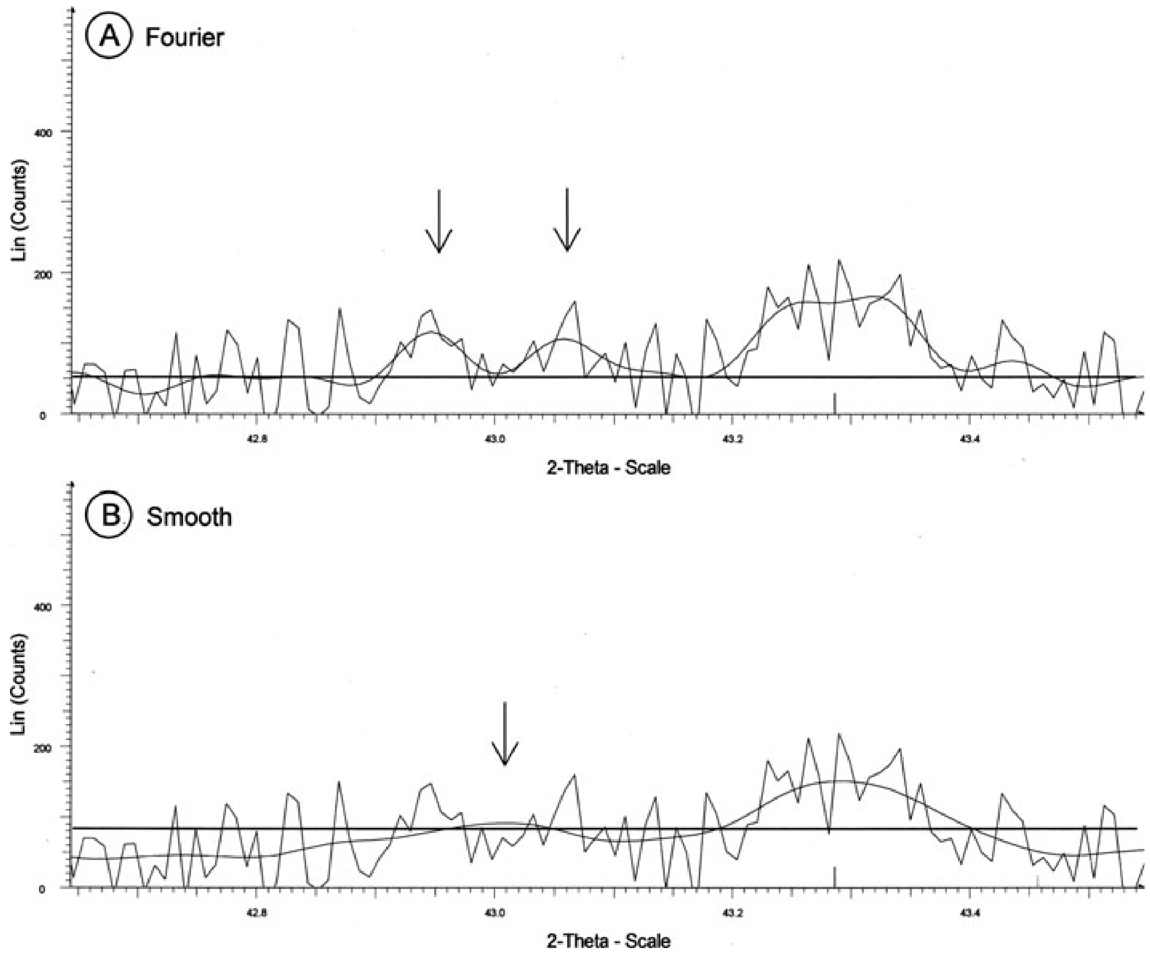

FIG. 15—*Comparison of mathematical smooth functions member.*

TABLE 7—*Composition and fulfillment of diffraction peak criterion of MDMA-HCl in the two organic mixtures.*

Sample Name	MDMA-HCl (wt%)	Matrix 1 (wt%)	Matrix 2 (wt%)	MDMA >4 Peaks	MDMA 2–4 Peaks	MDMA 1 Peak
MDMA3Matrix1-1_99	1	99		–	–	+
MDMA3Matrix1-2_98	2	98		–	+	+
MDMA3Matrix1-3_97	**3**	97		+	+	+
MDMA3Matrix1-4_96	4	96		+	+	+
MDMA3Matrix1-5_95	5	95		+	+	+
MDMA3Matrix1-10_90	10	90		+	+	+
MDMA3Matrix2-1_99	1		99	–	–	+
MDMA3Matrix2-2_98	**2**		98	**+**	+	+
MDMA3Matrix2-3_97	3		97	–	+	+
MDMA3Matrix2-4_96	4		96	+	+	+
MDMA3Matrix2-5_95	5		95	+	+	+
MDMA3Matrix2-10_90	10		90	+	+	+

TABLE 8—*Composition and fulfillment of diffraction peak criterion of the inorganic mixtures.*

Sample Name	$CaCO_3$ (wt%)	SiO_2 (wt%)	$CaCO_3$ >4 Peaks	$CaCO_3$ 2–4 Peaks	$CaCO_3$ 1 Peak
$CaCO_3SiO_2$-0.3_99.7	0.3	99.7	–	–	+
$CaCO_3SiO_2$-0.5_99.5	0.5	99.5	–	+	+
$CaCO_3SiO_2$-1_99	1	99	–	+	+
$CaCO_3SiO_2$-2_98	**2**	98	+	+	+
$CaCO_3SiO_2$-3_97	3	97	+	+	+
$CaCO_3SiO_2$-4_96	4	96	+	+	+
$CaCO_3SiO_2$-5_95	5	95	+	+	+
			SiO_2 >4 peaks	SiO_2 2–4 peak	SiO_2 1 peak
$CaCO_3SiO_2$-99.5_0.5	99.5	0.5	–	+	+
$CaCO_3SiO_2$-99_1	99	1	–	+	+
$CaCO_3SiO_2$-98_2	98	2	–	+	+
$CaCO_3SiO_2$-97_3	97	3	+	+	+
$CaCO_3SiO_2$-96_4	96	4	+	+	+
$CaCO_3SiO_2$-95_5	95	5	+	+	+

TABLE 9—*Results of blind tests for MDMA-HCl in organic mixture.*

Sample Name	MDMA-HCL (wt%)	Matrix 1 (wt%)	Matrix 2 (wt%)	Report Operator B
Blindtest A	3		97	++
Blindtest B	2		98	++
Blindtest D	1		99	o
Blindtest F			100	–
Blindtest E	3	97		+
Blindtest G	1	99		o
Blindtest H	2	98		o
Blindtest C		100		–

++, identification; +, detection; o, hint to a substance; –, not identified.

TABLE 10—*Results of blind tests for $CaCO_3$ and SiO_2 in inorganic matrix.*

Sample Name	$CaCO_3$ (wt%)	SiO_2 (wt%)	Report Operator B
Blindtest J	100	0	–
Blindtest K	99.6	0.4	+
Blindtest I	99	1	++
Blindtest M	0	100	–
Blindtest L	0.5	99.5	o
Blindtest N	1	99	o

++, identification; +, detection; o, hint to a substance; –, not identified.

In total, 15 laboratories took part and 13 laboratories provided their results. The samples tested consisted of:

- 25 mg Sicotan® (BASF, Ludiwigshafen, Germany), a yellowish pigment ($Ni_xSb_yTi_{1-x-y}O_2$) on rutile basis
- a 200 mg mixture of 10% Cu, 15% NH_4NO_3, 20% S_8, 35% Pb_3O_4, and 20% lactose hydrate
- a 300 mg mixture of 1/3 mannitol, 1/3 acetaminophen, and 1/3 caffeine

The samples were weighed with a Mettler balance and homogenized in an agate mortar for 1 h.

The Sicotan® sample was probably the most difficult one, because no matching pattern exists in the reference database ICDD PDF2. By the forensic scientific background and the elemental analysis information of Ni and Sb, an operator should be able to conclude that pure TiO_2 is a white pigment, so Ni and Sb are responsible for the yellowish color. Examination of the powder diffractogram with all that information should lead to the result of a rutile structure with additional nickel and antimony elements incorporated into the crystal lattice.

The proficiency test was evaluated by a point system (Table 11). As the Sicotan® was the most difficult sample, 0.5 points were given for the identification of rutile phase. Another half point was added if the elements nickel or antimony had been taken into account. Another full two points were given if the pigment Sicotan® was identified. Other samples were evaluated by giving two points for each identified phase. False-identified phases and not identified phases were not included in the point system as some laboratories would have received negative points.

The forensic laboratory of the German federal criminal police office, the Bundeskriminalamt, is Lab J. The secure phase identification and the "confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled" were proven once more, although the laboratory proficiency test shows that more tests are advisable.

Discussion

It is shown that the required parameters of trueness, selectivity, and specificity were fulfilled. Only phases below the detection limit could not be identified with PXRD although this problem can sometimes easily be solved by longer measurement time. Tested preparation errors showed to be nonsignificant for phase identification or are detected easily by an operator with scientific background, so in no case wrong phase identification should occur. No false-positive or false-negative results were reported at blind tests. Examination of background scattering showed that the generally

TABLE 11—*Results of the proficiency test.*

		Lab-Code												
Sample	Phases/Elements	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Rutile	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	Element Antimony	0.5	0.5	0.5	–	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	Element Nickel	0.5	0.5	0.5	–	0.5	0.5	0.5	–	0.5	0.5	0.5	0.5	–
	(Ti, Ni, Sb)O_2/*Sicotan® Gelb (Pigment Yellow 53)*	–	–	2	–	–	2	1.5	–	2	1.5	–	2	–
2	Lead oxide (Pb_3O_4)	2	2	2	2	2	2	2	2	2	2	2	2	2
	Sulfur	2	–	2	–	2	2	2	–	2	2	2	2	2
	Ammonium-nitrate	–	–	2	–	–	2	–	–	2	2	–	–	–
	Lactose hydrate	–	–	2	2	–	2	2	–	2	2	2	–	–
	Copper	–	–	2	–	2	2	2	–	2	2	2	1	2
3	Mannitol	2	–	2	2	2	2	2	2	2	2	2	2	–
	Acetaminophen	–	–	2	2	2	2	2	2	2	2	2	2	2
	Caffeine	–	2	2	2	2	2	2	2	2	2	2	2	–
… of max. 19.5 points		7.5	5.5	19.5	10.5	13.5	19.5	17.5	9	19.5	19	15.5	14.5	9
False-identified phase(s)		0	3	0	0	2	0	0	0	0	0	0	1	0
Not identified phase(s)		6	7	0	4	3	0	1	5	0	0	2	2	5

used peak criterion of three sigma is inadequate to test for the real presence of an X-ray diffraction peak. Recommendations for a more realistic criterion are given. The execution of a laboratory proficiency test showed that more tests should be carried out for scientific community.

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