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The identification of rimonabant polymorphs, sibutramine and analogues of both in counterfeit Acomplia bought on the internet

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ABSTRACT

Acomplia was ordered over the internet resulting in the delivery of counterfeit Acomplia and imitation products. The tablets were analyzed for the presence of rimonabant. Using LC-DAD–MSn the presence of effective quantities of rimonabant was confirmed in samples A–D. Samples A and D also contained traces of the rimonabant analogue NIDA-41020. Furthermore, NIR spectroscopy on the tablets indicated the presence of an unapproved rimonabant polymorph in samples C and D which was confirmed by Raman spectroscopy and X-ray diffraction (XRD). In sample E a low dose of sibutramine was found as well traces of N-desmethylsibutramine and bis-N-desmethylsibutramine.

Rimonabant was withdrawn from the market because of serious adverse events and lack of efficacy. The availability of poor quality products with rimonabant, impurities and unapproved polymorphs is worrying. Suspect weight-loss medicines should be screened for the presence of novel analogues.

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1. Introduction

At the end of 2008 the weight-loss drug Acomplia (rimonabant, Fig. 1) was withdrawn from the market because of unacceptable adverse events including depression and suicidal behavior [1]. Following the withdrawal by Sanofi-Aventis, Pfizer and Merck each discontinued the development of their cannabinoid-1 receptor (CB-1) antagonists: otenabant and taranabant [1]. Rimonabant was the first CB-1 antagonist employed in weight reduction and was also used – off-label – as an aid in smoking cessation [2]. The high commercial expectations for rimonabant were marked by pharmaceutical industry patenting at least eight different rimonabant polymorphs aimed at improving the pharmacokinetic characteristics [3,4].

Despite the lack of efficacy and the risk of serious adverse events the internet still offers ample opportunity to buy Acomplia or similar products. Whether these products are genuine, contain rimonabant or some other active pharmaceutical ingredient is uncertain. A 2002–2007 overview on illegal weight-loss products seized in the Netherlands showed that most products – regardless of the label claim – contained sibutramine or ephedrine [5]. Because Acomplia was marketed after the period covered in the overview it was considered in the interest of public health to investigate the product still on the market.

The Dutch Consumer Association had recently conducted an internet survey attempting to purchase medicines from unofficial sources on the internet without having a doctors' prescription. Among the delivered products were five different Acomplia-like products that by commission of the Ministry of Health were submitted to our laboratory for analysis.

2. Materials and methods

2.1. Samples and standards

Three different batches of reference Acomplia tablets and rimonabant powder were provided by Sanofi-Aventis (Basel). Reference NIDA-41020 and sibutramine HCl H_2O was purchased at Sigma–Aldrich (Zwijndrecht, The Netherlands). Five suspect products were brought in for analysis and were assigned letters A–E and their visual characteristics were recorded.

Formic acid (p.a.) and ammonium hydroxide (p.a.) were obtained from Merck (Darmstadt, Germany). Water was demineralised and filtered using a Millipak® 200 0.22-μm filter from Millipore B.V. (Amsterdam, The Netherlands). Acidified water was prepared by addition of 2 ml of formic acid to 1000 ml of water and adjusting the pH to 4.0 using NH_4OH.

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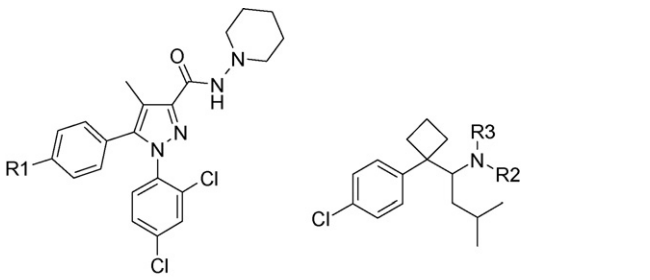

R1		R2	R3	
Cl	Rimonabant	Me	Me	Sibutramine
MeO	NIDA-41020	Me	H	N-desmethyl-sibutramine
		H	H	Bis-N-desmethyl-sibutramine

Fig. 1. Molecular structures of the CB-1 antagonists rimonabant, NIDA-41020 and of the serotonin–norepinephrine reuptake inhibitor sibutramine and two of its analogues.

2.2. LC-DAD–MSn

A composite was prepared of five tablets of each product. An amount of the tablet composite equivalent to half a dosage unit was extracted into 50 ml of MeOH in an ultrasonic bath for 20 min, centrifuged at 3200 rpm for 5 min. An aliquot (5.0 ml) of the supernatant liquid was diluted 100× using MeOH/acidified water 50:50), which solution was subsequently used for LC-DAD–MS analysis. All samples and solutions were filtered before use over 0.45 μm filter.

For chromatography and UV detection, a Surveyor instrumentation (Thermo Finnigan, Breda, The Netherlands) was used under the following conditions: XTerra MS® C18 analytical column (100 mm × 2.1 mm, 3.5 mm; Waters Chromatography B.V., Etten-Leur, The Netherlands); elution using solvent A—MeOH and solvent B—acidified water (v/v): 0–5 min isocratic (A/B = 50/50), 5–12 min gradient to A/B = 80/20, 12–28 min isocratic A/B = 80/20, 28–33 min: isocratic A/B = 50/50; flow rate at 0.25 ml min^{-1}; column temperature of 25 °C; injection volume of 20 μl; ultraviolet light detection from 200 to 450 nm.

Mass spectrometry was carried out in positive-ion mode using the ESI interface using an LCQ Advantage ion-trap mass spectrometer equipped with an ESI interface, operated by Xcalibur software version 1.4 (Thermo Finnigan B.V). Nitrogen was used as sheath gas (19 arbitrary units) and as auxiliary gas (10 arbitrary units). Source settings used: ion spray voltage 5.0 kV, capillary temperature 300 °C, capillary voltage 31 V, tube lens offset 55 V. MS1: mass range m/z 80–1000. MS^n: relevant MS1 ions were selected and fragmented using a collision energy of 35.00 arbitrary units using an isolation width of 5. Quantisation was performed at the UV_{max} of rimonabant (244 nm) and sibutramine (224 nm). Instrument conditions were checked using rimonabant standard working solution.

A three-point calibration curve was prepared in duplo for quantification: rimonabant; range: 30–50 μg/ml, precision $RSD_{rimonabant}$ = 0.4%, linearity R^2 = 0.9891, y = 616,272,951x + 16,492,373; sibutramine; range: 37–65 μg/ml, precision $RSD_{sibutramine}$ = 0.7%, linearity R^2 = 0.9992, y = 19,582x − 107.31.

2.3. Near infrared spectroscopy

A small reference library (30 spectra) of three different batches of genuine Acomplia was constructed for authentication of samples A–E in the current case and also for cases in the future. The methods used were identical to those we described earlier for Viagra tablets [6].

Product A–E, reference Acomplia tablets and rimonabant powder were analyzed using NIRS. Reference rimonabant (50 mg) was added twice to a powdered reference Acomplia tablet to detect the characteristic absorption bands of rimonabant in the second derivative NIR spectra.

2.4. Raman spectroscopy

To investigate the presence of rimonabant polymorphs in samples C and D they were subjected to Raman spectroscopy. Genuine Acomplia and reference rimonabant were used for comparison. Raman spectroscopy carried out using an FT-Raman instrument: Spectrum2000 Perkin Elmer Co. Ltd. equipped with an Nd:YAG laser. Before the measurement the top layer of the tablets was scraped off and the freshly prepared surface was examined. In case of the reference rimonabant powder a few granules were measured in the powder sample holder. The settings were 350 mW, 4 cm^{-1} spectral resolution, 350 scans per spectrum.

2.5. X-ray diffraction

To identify the rimonabant polymorphs X-ray diffraction experiments were carried out and the results were to be compared to data from patent literature. For all X-ray experiments Bruker AXS D8 Advance powder diffractometer was used. A special configuration consisting of X-ray tube, multilayer X-ray mirror (Göbel mirror), sample holder and gaseous, position sensitive Våntec detector was applied. Cu Kα radiation and a θ–θ scan mode were used to collect diffraction data. From the samples involving coated tablets the thin coating was removed to avoid the influence of unimportant and highly diffracting excipients (e.g. titanium dioxide—TiO_2). Then, samples were grounded in an agate mortar and the resulted powders were moved to the sample holders. The diffraction pattern for reference rimonabant powder was also collected. Diffraction patterns were collected in Bragg angles 2θ range 3–60° and results were compared to data on rimonabant polymorphs in patent literature and to a commercial database for the identification of excipients [7].

Table 1
Analysis results for the five samples.

Sample	Product name	Quantity received	Lot no./exp.	Identity[a]	Polymorph	Dose (mg)
A	Rimonabant 20 mg	3 × 30 tablets	22301/07-2009	Rimonabant	2	19.0
				NIDA-41020	–	Traces
B	Riomont	3 × 10 tablets	K82349/06-2010	Rimonabant	2	19.1
C	Slimbant	3 × 10 tablets	CT/8/754/07-2010	Rimonabant	1	19.7
D	Rimonabant tablets	4 × 15 tablets	RB2-0001 9/08-08 12	Rimonabant	1	16.6
				NIDA-41020	–	Traces
E	Acomplia	1 × 29 tablets	–	Sibutramine	–	2.0
				N-desmethylsibutramine	–	Traces
				Bis-N-desmethylsibutramine	–	Traces

[a] Genuine Acomplia contains 20 mg rimonabant polymorph 2.

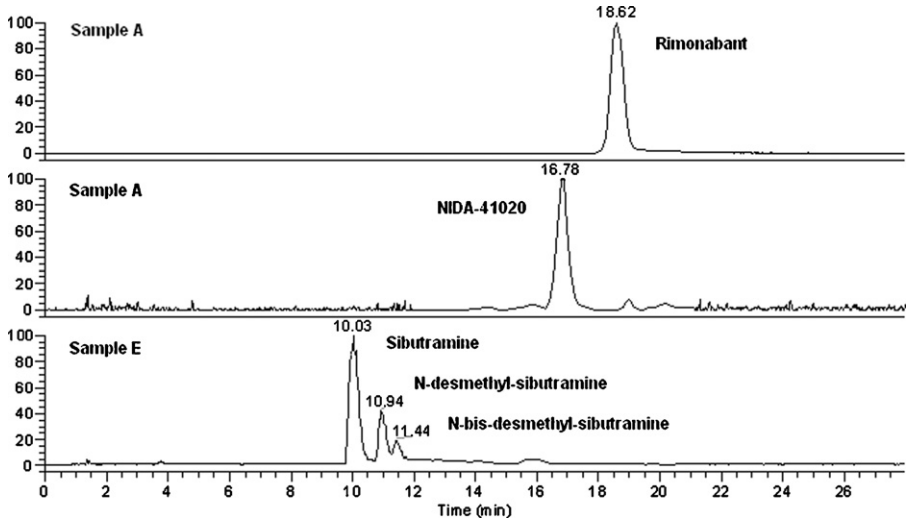

Fig. 2. Filtered full scan traces of rimonabant (*m*/*z* 463–467) and NIDA-41020 (*m*/*z* 459–461) in sample A and of sibutramine and analogues in sample E (*m*/*z* 252–254, 266–268, 280–282).

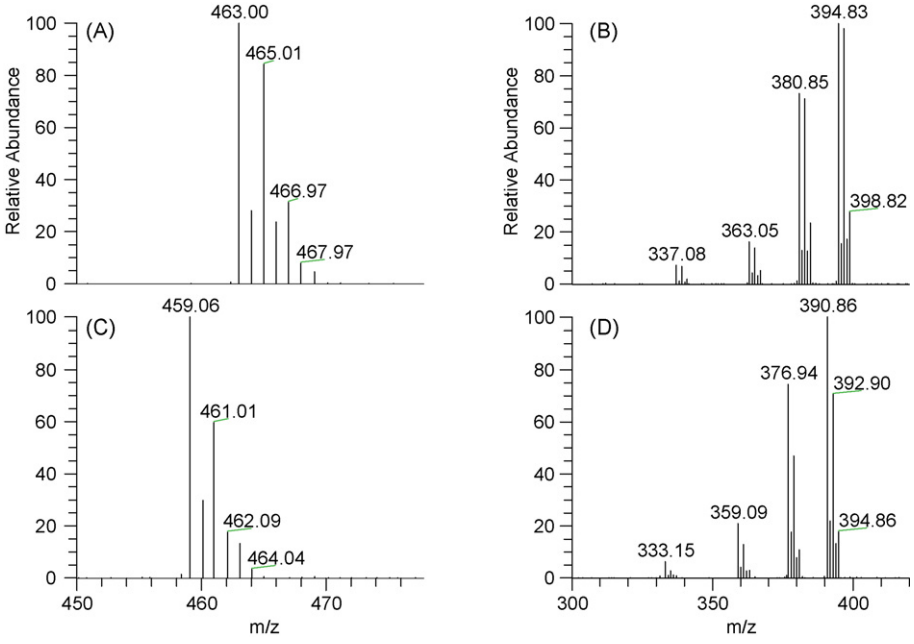

Fig. 3. Full scan MS data for rimonabant (A) and NIDA-41020 (C) and, respectively, their MS2 fragments (B) and (D).

3. Results and discussion

3.1. Samples

Samples A, C and D were received as blister packs only. Sample B consisted of a cardboard box with three blister packs. The tablets of samples A–D were unmarked, white and round with exception of a score line for sample C. Sample E was a plastic grip bag with loose tablets shaped as teardrops marked with an '20' imprint resembling the appearance of genuine Acomplia tablets. Only samples A and B were received with a patient information leaflet of which the contents were not assessed. Lot numbers and expirations dates are given in Table 1.

3.2. LC-DAD–MS

Fig. 2 shows an example of a filtered full scan trace of the chromatogram of samples A and E. The identity of rimonabant in samples A–D and of sibutramine in product E was confirmed by RT, UV-absorbance, MS1 and ion ratio's in MS2. Dosages of rimonabant and sibutramine were calculated using the three-point calibration curve (Table 1). The quantities of rimonabant found in samples A–C are in agreement with the declared dosage. Sample D contained only 83% of the declared value.

In samples A and D traces of the same unknown impurity were detected by MS. The MH^+ ion and the MS2 fragments were consistently four mass units under those of rimonabant (Fig. 3). These findings indicated a strong resemblance in molecular structure between rimonabant and the unknown impurity. The observed isotope patterns suggested the presence of two chlorine atoms rather than three as in rimonabant. Therefore, the difference in molecular mass and isotopes is best explained by the replacement of a chlorine atom for a methoxy-group. A literature search showed such a compound was reported as a potent CB-1 antagonist with the code name NIDA-41020 (Fig. 1) [8,9]. Using reference NIDA-41020 the identity of the unknown component in samples A and D was

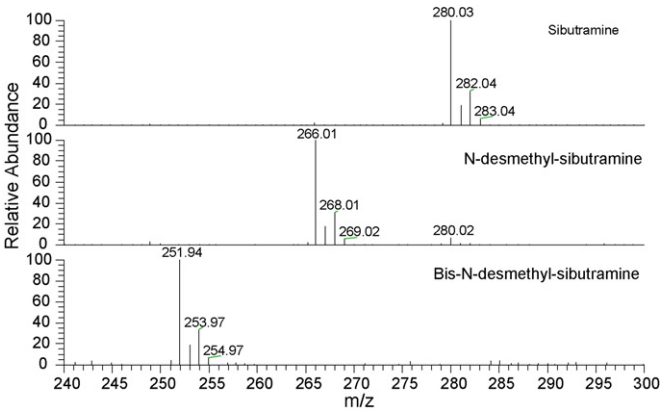

Fig. 4. Full scan MS data for sibutramine and the two analogues in sample E.

confirmed by RT, MS1 and ion ratios in MS2. The levels of the impurity relative to rimonabant were estimated at 0.43% (sample A) and 0.28% (sample D) using the full scan peaks for NIDA-41020 (*m/z* 459–463) and rimonabant (*m/z* 463–467). NIDA-41020 is not an impurity that normally can be expected to be formed in the synthesis of rimonabant [10]. Therefore, its presence is rather explained by the use of impure starting materials or poor production hygiene.

In sample E two components related to sibutramine were detected with MH^+ ions 14 and 28 mass units under that of sibutramine (Fig. 4). Their MH^+ isotope patterns suggested the presence of one chlorine atom and their fragmentation in MS2 could not be distinguished from that of sibutramine (*m/z* 179 (10%), 153 (58%), 139 (100%), 125 (18%)). These findings are consistent with N-desmethylsibutramine and bis-N-desmethylsibutramine which are a known active metabolites of sibutramine and are also frequently identified in weight-loss products [11]. In absence of a reference standard their identity was not confirmed.

3.3. Near infrared spectroscopy

The NIR spectra of the samples A–E were all different from the reference spectra of Acomplia tablets. The spectra of the five tablets analyzed of each sample were mutually equal. Differences are attributed to differences in the total composition (active ingredient + excipients).

Similarities in NIR spectra were observed for samples A and B and for samples C and D. NIR spectra of sample E were unlike any other which is in line with the LC-DAD–MS results. Standard addition measurements showed specific absorption maxima of rimonabant at 6467, 6034, 5974, and 4836 cm^{-1}. Using the isolated maxima at 6467 and 4836 cm^{-1} the presence of rimonabant could be confirmed in the second derivative spectra for samples A and B. However, these maxima were not observed for samples C and D thus no rimonabant could be detected (Fig. 5). For sample C and D this was inconsistent with the LC-DAD–MS results indicating the potential use of a different rimonabant polymorph. The results for sample E were omitted from Fig. 5 for reasons of legibility.

3.4. Raman spectroscopy

To confirm the presence of a different rimonabant polymorphs Sample C and D were investigated using Raman spectroscopy. The recorded Raman spectra showed that the bands observed for the reference rimonabant in the area of 1600, 1428 and 746 cm^{-1} well matched the bands observed for samples C and D and genuine Acomplia. Especially the bands in the area of 1600 cm^{-1} related to C=C double bonds can be therefore assigned to rimonabant.

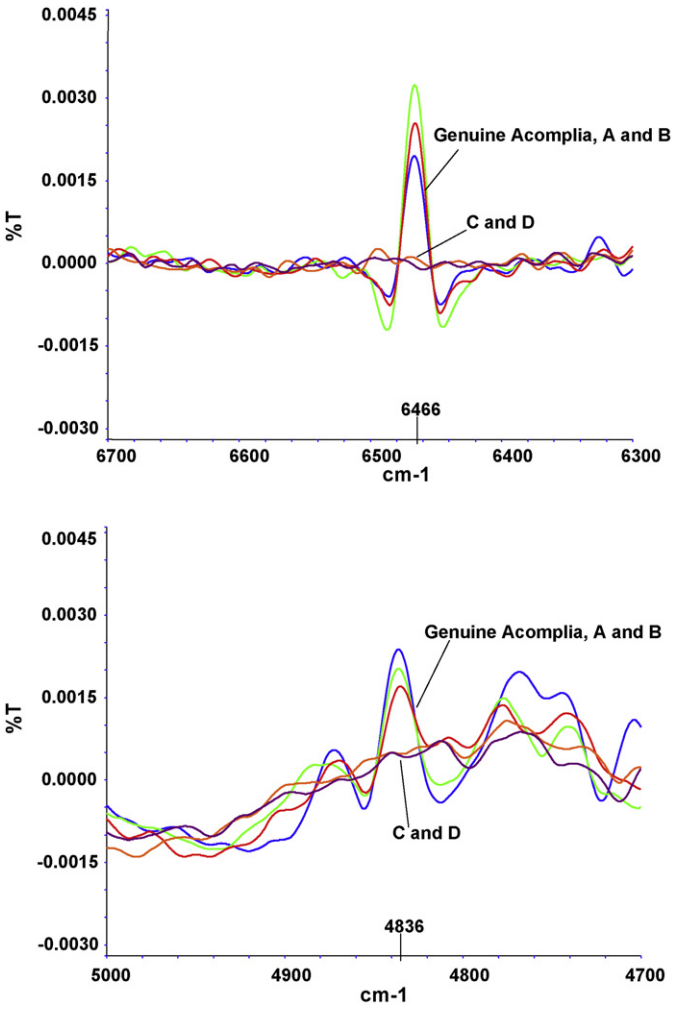

Fig. 5. Zoom in on two areas of NIR spectra recorded for samples A–D and genuine Acomplia. Specific signals for reference rimonabant are at 6466 and 4836 cm^{-1}.

Zooming in to the 1600 cm^{-1} region two similar bands were observed in samples C and D, genuine Acomplia and reference rimonabant. However, the two bands for samples C and D were slightly different from those for genuine Acomplia and reference rimonabant in peak shape and exact peak position (Fig. 6). These differences can only be explained by a difference in conformation, hence the presence of a different rimonabant polymorph.

The bands around 1680 cm^{-1} are assigned to C=O vibrational states and are considered as not sufficiently significant for rimonabant (could also originate from the excipients). There are no Raman spectra for comparison, however, reported IR absorption bands for rimonabant polymorph 2 match the 1683 cm^{-1} bands from genuine Acomplia and reference rimonabant, and the 1668 cm^{-1} bands for samples C and D match the reported IR bands of rimonabant polymorph 1 [3]. Because no firm conclusions could be drawn from this it was decided to further investigate the polymorphism using X-ray diffraction (XRD).

3.5. X-ray diffraction

The initial patent on rimonabant polymorphism by then Sanofi-Synthelabo was followed by many patents on other polymorphs by competing pharmaceutical industry [3,4]. These patents provide evidence of a novel polymorphic form by demonstrating their X-ray diffraction pattern is different from those already known. For patenting purposes the pure substance is used for XRD. However,

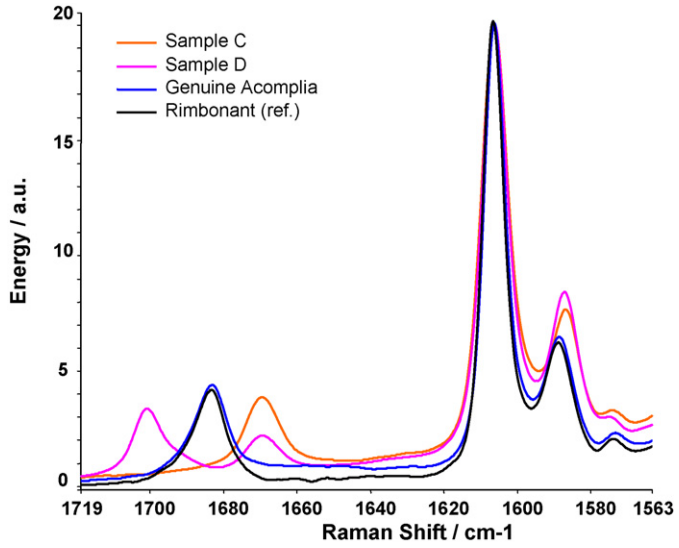

Fig. 6. Zoom in on the Raman spectra recorded for samples C and D, genuine Acomplia and reference rimonabant (1563–1719 cm^{-1}).

is has also been shown that XRD can be useful for determining API polymorphism and excipients in tablets [12]. Therefore, samples A–D, genuine Acomplia and reference rimonabant were investigated using XRD.

Fig. 7 shows the X-ray diffraction patterns for all tablets to be different. For samples A and B and genuine Acomplia two broad background maxima were observed in the 2θ range 13.5–18° and 18.5–24.5° which are characteristic for microcrystalline cellulose. The sharp peaks in this area could all be assigned to the presence of lactose monohydrate. In samples C and D there is no indication for the presence of lactose monohydrate. Instead, comparison of the maxima observed at $2\theta \approx 12.5°$, 20.8°, 29.1°, 31.1° and 33.2° suggests the presence of gypsum.

Because the content of excipients is much higher than that of the API, the best way to discern between different polymorphs is to compare high intensity, low angle reflections of the samples with characteristic diffraction patterns listed in patent literature. The Sanofi-Synthelabo patent describes genuine Acomplia should contain rimonabant 'polymorph 2' which is characterized by peaks at $2\theta \approx 5.1°$, 10.1° and 10.8°. Indeed, such peaks can be distinguished for samples A and B and for genuine Acomplia (Fig. 8). Other characteristic peaks for polymorph 2 are either hidden in a broad microcellulose background (e.g. $2\theta \approx 15.2°$) or are superimposed on

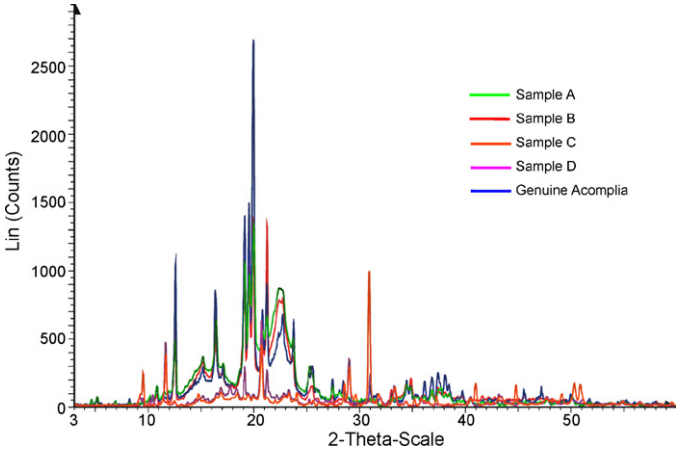

Fig. 7. Diffraction patterns of samples A–D and genuine Acomplia.

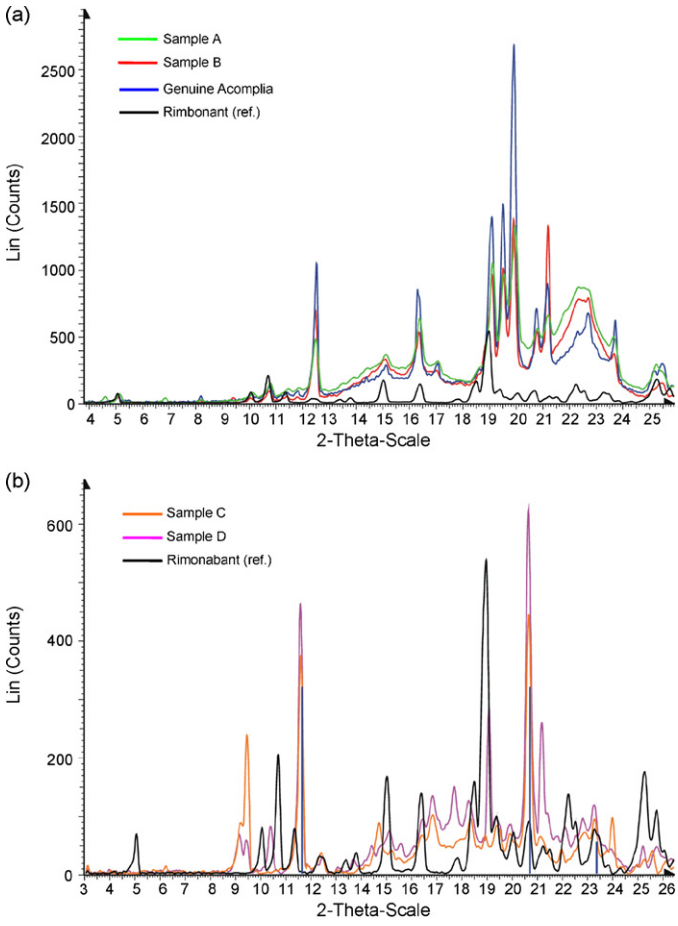

Fig. 8. (a). Lower angle part of the diffraction patterns for samples A and B, genuine Acomplia and reference rimonabant. (b) Lower angle part of the diffraction patterns for samples C and D and reference rimonabant.

the lactose high maxima (e.g. $2\theta \approx 19.1°$). Several low angle peaks in the diffraction patterns of samples A and B are not present in genuine Acomplia or reference rimonabant and are attributed to excipients.

The lower angle part of the diffraction patterns for samples C and D confirms they contain the same rimonabant polymorph (Fig. 8) and are different from polymorph 2. Both samples are characterized by diffraction maxima at 2θ angles of ca. 9.15°, 11.6°, 12.3°, 16.4°, 16.86°, 18.38°, 19.4°, 20.7°, 21.3° and 22.9° which are characteristic for rimonabant polymorph form 1 [3]. Interfering peaks from the matrix were only observed around $2\theta \approx 12.5°$ and 20.8° which were attributed to gypsum. Therefore, there is strong evidence of the presence of rimonabant polymorph 1 in samples C and D. In absence of a reference standard this could not be verified.

4. Conclusions

The results of this study show that the use of only chromatographic techniques is insufficient when investigating the active ingredients of illegal medicines. In this study spectroscopic and diffraction techniques have added valuable information on polymorphism. The analysis results underscore the risks of purchasing medicines on the internet from unofficial sources. First of all, the actual presence of rimonabant in efficacious amounts in samples A–D is worrying because it was found unsafe even under the supervision of physicians. Second, there is strong evidence of the presence of rimonabant polymorph 1 in samples C and D. As rimonabant polymorphs have a demonstrated different pharmacokinetic

profile their clinical performance may be different. Therefore, the use of such unapproved rimonabant polymorph may have consequences for the occurrence of the dangerous side effects. Third, the presence of the rimonabant analogue NIDA-41020 in samples A and D demonstrates a poor manufacturing practice. In the worst case the API manufacturer is also involved in the production of NIDA-41020 and does not thoroughly clean the equipment between batches. Interestingly, samples A and D both have this impurity but contain a different rimonabant polymorph. Fourth, the only counterfeit among the samples contained sibutramine and two of its analogues but not rimonabant. Even though the sibutramine dosage was not that high it underlines that counterfeiters seem prepared to use whatever weight-loss drug available, regardless of its purity.

Consumers purchasing Acomplia-like products from unofficial sources on the internet have no guarantees of what exactly they will receive. The fact that APIs were found in all products shows that the manufacturers – even counterfeiters – are aiming at returning customers by providing substandard but efficacious products.

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