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USE OF HYPHENATED LC-MS/MS TECHNIQUE FOR CHARACTERIZATION OF IMPURITY PROFILE OF QUETIAPINE DURING DRUG DEVELOPMENT

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Abstract: As a part of an integrated quality concept in drug development, the multidimensional evaluation of impurity profiles by LC-MS/MS is presented for quetiapine – an active pharmaceutical ingredient (API). LC-UV is commonly employed for the determination of impurities and degradation products. In this work LC-MS/MS technique is proposed as a modern alternative for the characterization of these compounds. The use of this technique allowed to develop methods for the separation and identification of the impurities resulting from both, synthesis and degradation processes.

Keywords: LC-MS/MS, impurity profile, quetiapine

Quetiapine (2-[2-(4-dibenzo[b,f]1,4-thiazepin-11-yl-1-piperazinyl)ethoxy]ethanol fumarate (Fig. 1) is an antipsychotic drug belonging to the class of dibenzothiazepines. It is used as a hemifumarate salt. Quetiapine is an antagonist of a broad range of neurotransmitter receptors. It is an atypical antipsychotic drug used for the treatment of schizophrenia and other psychotic syndromes (1–3).

One of the most important challenges of current life sciences research is the comprehensive and accurate analysis of various forms of the matter. Therefore, it is very important to develop highly specialized analytical tools allowing a simple, quick and accurate determination of impurities in water, soil or in active pharmaceutical ingredients (API). An API should be characterized by properties which guarantee its safety and efficacy. The pharmaceutical industry, as the main producer of API's, is responsible for their quality. There are three criteria of the quality assessment of an API: product identity, assay criterion and purity criterion referring to impurities.

Impurity is considered as any by-product occurring in the active substance, which needs to be controlled, even if it is completely neutral or its pharmacological properties are better than that of the API's (4). According to another definition, it is any ingredient of the new API which is not a chemical entity defined as an API (5). There are different sources and types of impurities which can occur in an API. Pharmaceutical impurities consist of process-related impurities and degradation products – both to be controlled as API constituents. Many potential impurities come from the manufacturing process of API and consist of starting materials (6), intermediates, reagents, catalyst (7), solvents (8) and by-products. These potential impurities should be studied in order to determine the mechanisms of controlling the manufacturing process of API with the aim of, on one hand, eliminating them, and on the other, including them in the specification for routine control at accepted levels. Early understanding of the nature and mechanism of impurity formation enables to incorporate a control strategy into the manufacturing process. It is also important to study impurities in starting materials used in the API synthesis. There are only very few reports on this source of impurities in APIs, however, the risk of contamination of API in this way is very high (9).

Impurity control in drug products is the primary goal of drug development (10). Stringent international regulatory requirements regarding impurities have been discussed for several years and outlined in the International Conference on Harmonization (ICH) Guidelines Q3A(R), Q3B(R) and Q3C (5, 11, 12). All these aspects and requirements indicate a new trend of studying an impurity profile rather than a purity profile. The definition of the impurity profile of the new drug products was reported by ICH as "description of known and unknown impurities present in the new DS". It is the common name of analytical activities with the aim of detecting, identifying or elucidating the structure and quantitative determination of organic and inor-

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ganic impurities as well as residual solvents in APIs. Of the three groups of impurities mentioned above the estimation of the profile of organic impurities is the most challenging and interesting task.

A variety of techniques are available for monitoring impurities (13). These can include spectroscopic and separation methods or a combination of both. The ideal method should be specific, selective, precise, accurate and highly sensitive. Nowadays, it is becoming possible, due to the use of technologies coupled with mass spectrometry, which allow to determine the identity, purity and assay of studied substances (14, 15) simultaneously. Impurity monitoring in APIs is often limited to the analysis of the known compounds, whereas the unknown compound analysis, as more complicated one, requires instrumental methods to obtain structural information. Liquid chromatography-mass spectrometry (LC-MS) has become the primary approach for the identification of low-level impurities in samples resulting from synthesis or from degradation of APIs. Full scan and product ion scan analysis, providing molecular weight information and fragmentation data, respectively, offer rich structural information on candidate structures. There are many strategies to identify impurities (15). The one described in this article involves the use of the parent drug itself as a template for the interpretation of the unknown structures. First, the parent drug is analyzed with LC-MS. The retention time and molecular weight information are obtained. Using LC-MS/MS, the product-ion analysis of the parent drug is obtained, and specific product ions and neutral losses are assigned to the substructures of the molecule. The MS/MS identification strategy is based on the assumption that much of the parent drug structure will be retained in the impurities or decomposition products. The product-ion mass spectrum of the parent drug and fragmentation pattern of the parent drug are used as the templates for the identification of the unknown structure.

In this paper the typical experimental setup is described that is used routinely for LC-MS/MS experiments. Representative applications of the strategy are also given in structural elucidation of impurities in API's. The multidimensional evaluation of impurity profiles by LC-MS/MS coupling is illustrated on quetiapine as an API.

EXPERIMENTAL

Chemicals

The active substance – quetiapine (QT6), as well as process-related impurities (QT2, QT3, QT3z, QT4, QT4z, QT5, QT5z) of > 99.5 % purity were synthesized at Pharmaceutical Research Institute, Warszawa, Poland. Chemicals of an analytical grade were used for the study. LC grade acetonitrile and methanol were purchased from Merck, Darmstadt, Germany. Analytical reagent grade ammonium acetate was obtained from Fluka, Steinheim, Germany. High purity water was prepared by a Millipore Milli-Q plus water purification system.

Equipment and methods

Mass spectrometry (MS)

The MS detector was used for the identification of process impurities and degradation products. The MS analysis was performed on a MS/MS mass spectrometer model 3200 Q TRAP (Applied Biosystems, Concord, Ontario, Canada). The quadrupole/linear ion trap is a hybrid system in which the final quadrupole can operate as a conventional mass filter or as a linear IT (ion trap) with an axial ion inject. The analyses were performed in positive and negative ionization modes with Turbo Ion Spray interface under the following conditions: ionspray source voltage (IS), 4500 V; curtain gas (CUR), 25 (arbitrary units); declustering potential (DP), 20 V; entrance potential (EP), 10 V; collision energy (CE), 20. Nitrogen served as a turbo-gas and collision gas. The analyses were performed in an Enhanced MS (EMS) mode, in an Enhanced Product Ion (EPI) mode, MS^3 mode and Multiple Reaction Monitoring (MRM) mode. The following transitions were monitored, each with the 20 ms dwell time: 314/87, 296/210, 384/253, 202/124, 322/228, 540/113, 429/202, 429/124, 505/253, 228. In this study the LightSight™ software was used for automatic generation, identification and characterization of expected and unexpected impurities in the samples.

Chromatographic system (LC)

Shimadzu LC – 20A system was used for the method development, process impurities and forced degradation studies. LC – 20A is equipped with a binary gradient pump (LC-20AD), mixer, five channel vacuum degasser, autosampler with cooling (SIL-20AC), thermostat for column (CTO-20AC) and UV/VIS detector (SPD-20A). The output signal was monitored and processed using the Analyst software.

Chromatographic conditions

Waters Symmetry-C18 chromatographic column was used (250 mm × 4.6 mm, 5 μm). The

Table 1. Description of stress testing experiments.

No. of exp.	Conditions of stress reaction	Sample Preparation
Impact of acidic environment		
1	0.1 M HCl/H_2O reflux	100 mg of quetiapine was dissolved in 10 mL of 0.1 M HCl/H_2O. After reaction 100 µL of this mixture was dissolved in 1 mL of methanol.
Impact of H2O2		
2	3% H_2O_2 reflux	100 mg of quetiapine was dissolved in 10 mL of 3% H_2O_2/H_2O. After reaction 100 µL of this mixture was dissolved in 1 mL of methanol.

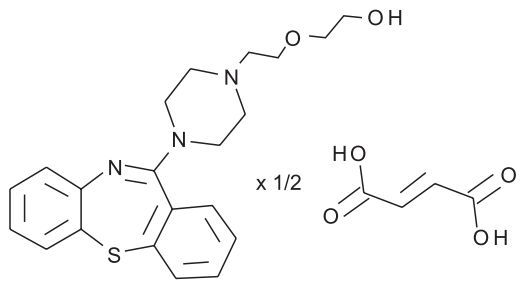


Figure 1. Structure of quetiapine

mobile phase contained a mixture of buffer (phase A) and acetonitrile (phase B) in the gradient system. The gradient HPLC separations started with 40% phase B (over a period of 15 min.), which was changed into 90% phase B over a period of 30 min. Elution continued for 10 min. using solvent B, which was afterwards changed back to solvent A over another 5 min. Equilibration of the column with solvent A for 10 min was performed before injecting of a new sample. The buffer solution consisted of 10 mM ammonium acetate with pH adjusted to 7.0 using potassium hydroxide solution. The flow rate of the mobile phase was 1.0 mL/min. The column temperature was maintained at 23°C and the detection was monitored at a wavelength of 240 nm. The injection volume was 20 µL, methanol was used as a diluent.

Preparation of solutions

Preparation of standard solutions

A stock solution of quetiapine hemifumarate (1.0 mg/mL) was prepared in methanol. A stock solution of synthesized impurities as mixtures at a concentration of 1.0 mg/mL was also prepared in methanol. Working solutions were prepared from the respective stock solutions.

Preparation of forced degradation solutions

The description of stress testing experiments is given in Table 1.

RESULTS AND DISCUSSION

Identification of the impurities from the synthesis

The impurities resulting from the synthesis were identified using an MS detector. The obtained mass and fragmentation spectra were used. The identified impurities were synthesized for the comparison with the compound recorded in the tested samples. Complete identification of the impurities (QT2, QT3, QT3z, QT4, QT4z, QT5z, QT5) was described in the article of Stolarczyk et al. (16). These impurities were used to develop chromatographic and MS detection conditions. The complete identification of impurities occurring in the quetiapine samples was performed by the comparison of retention times, UV spectra and MS spectra with the ones for the synthetic impurities. The LC-MS/MS method developed and described in this article was used in the analyses. Figure 2A shows typical UV chromatogram of the mixture containing quetiapine and its impurities. To prove the selectivity of the method, a commercial sample of impurity L was used. This impurity is also resulting from quetiapine stress test in the acidic conditions. Figure 2B shows a typical MRM chromatogram of the mixture containing quetiapine and its impurities.

As a result of the quetiapine samples analysis, an additional impurity (imp. H) was identified at the level of = 0,1 %. This impurity was eluted between imp. L and quetiapine (Fig. 3). A pseudomolecular ion at m/z 340 is recorded in the mass spectrum of imp. H. Therefore, the mass of the imp. H is 339 Da, which is 44 Da less than quetiapine mass. The odd molecular mass of the imp. H suggest the odd nitrogen atom number in the molecule, which in turn suggest the presence of the stable piperazinyl-dibenzothiazepine ring in the impurity structure.

On the basis of the above information it was concluded that this impurity might be 2-[4-(dibenzo[b,f][1,4]thiazepin-11-yl)piperazin-1-yl]ethanol. The fragmentation spectrum of imp. H (Fig. 4) shows many fragmentation ions, which are the same

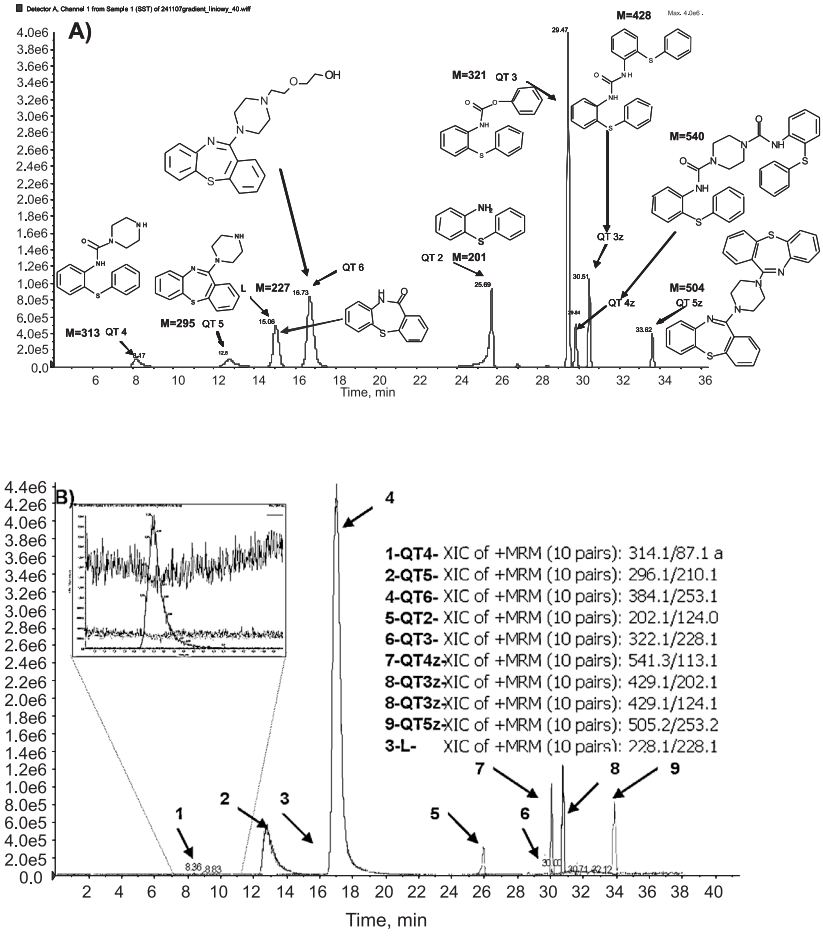


Figure 2. Chromatograms of the mixture containing the synthesized impurities and quetiapine: **A**) UV detection; **B**) MS detection, MRM mode

as in the quetapine spectrum e.g., m/z: 210, 221, 247, 253, 279. The hypothesis is supported by the presence of the ion m/z 114, which structure is present in the fragmentation spectrum of the imp. H.

The imp. H does not result directly from the synthesis. It is probably created as a result of alkylation of the intermediate QT5 using 2-[2-chloroethoxy]ethanol (CEE) as the alkylation agent. 2-Chloroethanol is the impurity of CEE. The method developed also enables to control quetiapine purity depending on the purity of CEE used.

Identification of degradation impurities in forced degradation studies

Stress studies on quetiapine hemifumarate under different conditions suggested that quetiapine is very stable under alkaline conditions, practically stable under neutral condition and photo stable. Quetiapine is very labile under acidic and oxidation conditions.

The stress tests conducted in order to identify the degradation impurities in quetiapine involved generating of main degradation products, at least at the level suitable for their identification. Therefore, this work presents the results after the acidic hydrolyses and following the treatment of quetiapine with peroxide in the conditions which allowed to obtain the main degradation product. Untreated quetiapine was a reference sample.

The first step of the study leading to the determination of the unknown degradation product structure was the identification of pseudomolecular and adduct ions. The latter one confirmed the determination of pseudomolecular ions. Cluster ions were subsequently observed in the spectra of almost all impurities, e.g., $[2M+H]^+$. For impurities B, E, F, G, clusters with solvents $[M+CH_3COO]^-$ type were observed in negative ionization. The next step was to record fragmentation spectra. The obtained

results of the MS analysis of the studied quetiapine degradation impurities are presented in Table 2.

Oxidative conditions: Quetiapine hemifumarate showed significant sensitivity towards hydrogen peroxide. Six major peaks are observed on the chromatogram of the oxidized sample (Fig. 5A). The prominent degradation was observed at RT ~ 2.8 min (imp. B), at RT ~ 5.3 min (imp. E), at RT ~ 3.6 min (imp. C), at RT ~ 4.6 min (imp. D), at RT ~ 2.2 min (imp. A) and at RT ~ 8.9 min (imp. F).

The (+) ve electrospray ionization (ESI) spectrum of the D and E impurity showed peaks at m/z 400 and 422, for protonated and sodium compounds, respectively, which accounts for 16 Da rise compared to quetiapine. These impurities had different retention times, therefore they are different compounds of 399 Da, formed throught the single oxi-

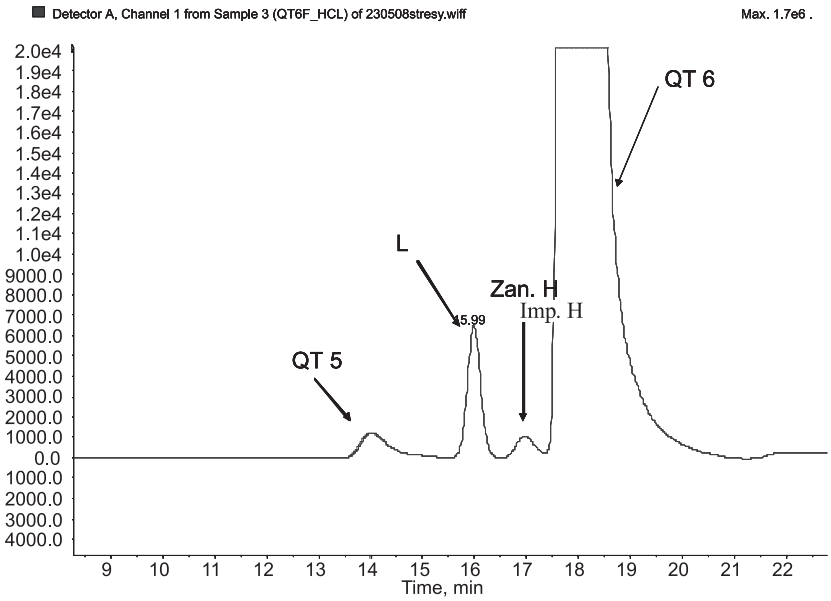


Figure 3. UV chromatogram of quetiapine with the imp.H and imp. L

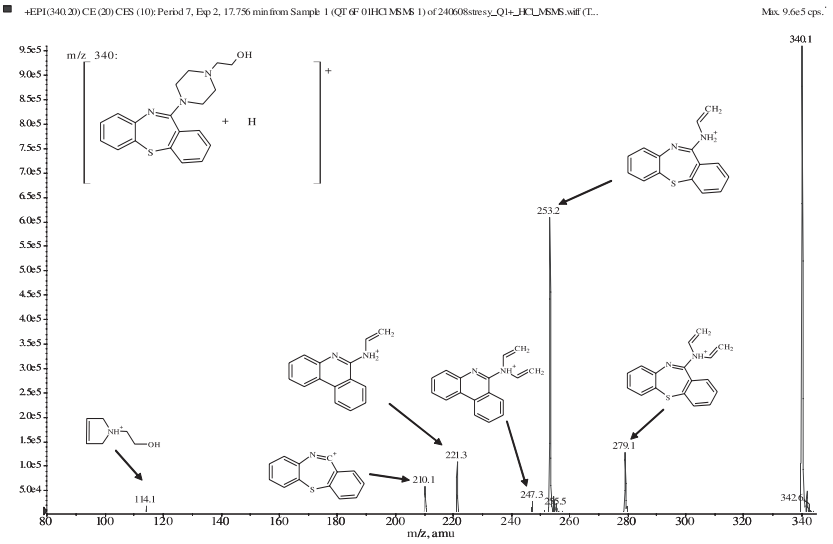


Figure 4. Fragmentation spectrum (EPI) obtained for imp. H and the proposed structures of characteristic fragments, CE = 20 V

Table 2. MS summary results of the impurity degradation analysis of quetiapine.

Oxidative conditions, (3% H_2O_2)			
Imp./R.T. (min.)	MS, ions, m/z		Mass shift /proposed transformation
	Q1	EPI (rel. int., %)	
A / 2.2	+ve: 416 $[M+H]^+$, 831 $[2M+H]^+$	416: 368 (13), 350 (5), 324 (4), 311 (6), 295 (10), 285 (16), 267 (8), 247 (16), 243 (18), 221 (13), 219 (15), 207 (38)	+32 Da / double oxidation (2O)
B / 2.8	+ve: 416 $[M+H]^+$, 438 $[M+Na]^+$, 831 $[2M+H]^+$, 853 $[2M+Na]^+$ -ve: 414 $[M-H]^-$, 474 $[M+CH_3COO]^-$, 889 $[2M+CH_3COO]^-$	416: 368 (15), 350 (6), 324 (4), 311 (7), 295 (12), 85 (20), 267 (10), 247 (20), 243 (22), 221 (17), 219 (17), 207 (40)	+32 Da / double oxidation (2O)
C / 3.6	+ve: 432 $[M+H]^+$, 454 $[M+Na]^+$, 863 $[2M+H]^+$, 853 $[2M+Na]^+$	432: 415 (19), 402 (2), 388 (3), 326 (2), 311 (11), 285 (9), 283 (5), 271 (9), 259 (1,5), 242 (3), 177 (1), 133 (2)	+48 Da triple oxidation (3O)
D / 4.7	+ve: 400 $[M+H]^+$, 422 $[M+Na]^+$, 799 $[2M+H]^+$, 821 $[2M+Na]^+$	400: 356 (4), 324 (6), 311 (7)	+16 Da / oxidation (O)
E / 5.4	+ve: 400 $[M+H]^+$, 422 $[M+Na]^+$, 799 $[2M+H]^+$, 821 $[2M+Na]^+$ -ve: 398 $[M-H]^-$, 458 $[M+CH_3COO]^-$,	400: 352 (75), 296 (8), 269 (40), 248 (22), 221 (90), 158 (16)	+16 Da / oxidation (O)
F / 8.9	+ve: 416 $[M+H]^+$, 438 $[M+Na]^+$, 831 $[2M+H]^+$, 853 $[2M+Na]^+$ -ve: 414 $[M-H]^-$, 474 $[M+CH_3COO]^-$,	416: 355 (6), 312 (40), 286 (94), 243 (2), 158 (7), 132 (4)	+ 32 Da / double oxidation (2O)
Acidic conditions, (0.1 M HCl)			
G / 5.4	+ve: 402 $[M+H]^+$, 424 $[M+Na]^+$, 803 $[2M+H]^+$, 825 $[2M+Na]^+$ -ve: 400 $[M-H]^-$, 460 $[M+CH_3COO]^-$,	402: 384 (4), 340 (2), 309 (13), 254 (2), 228 (84), 175 (45), 137 (1,5), 113 (5,5)	+ 18 Da / water addition

dation of quetiapine. The m/z 352 ion is observed on the fragmentation spectrum of the imp. E, which reflects the loss of neutral 48 Da molecule, i.e., SO. It was concluded that the imp. E might be a sulfoxide. Identification of the main ions, observed on the fragmentation spectrum of imp. E, is presented in Table 3. The compound D can contain an OH group in one of its rings but the N-oxidation of one of the nitrogen atoms is more likely. The results obtained by MS did not allow to identify which of the nitrogen atoms was oxidized.

The (+) ve electrospray ionization (ESI) spectrum of the A, B and F impurity showed peaks at m/z 416 and 438 for protonated and sodium compounds, respectively. This accounts for 32 Da rise compared to quetiapine. These impurities had different retention times, therefore they are different compounds of 415 Da, formed through the double oxidation of quetiapine. An m/z 368 ion is observed on the fragmentation spectrum of the imp. A, which reflects the loss of neutral 48 Da molecule which can be SO. On the fragmentation spectrum of the imp. A, two ions at m/z 350 and 267 were observed which reflect the loss of water molecule from suitable fragments. It was concluded that the imp. A is a sulfoxide with an additional OH group. The OH group can be in one of the rings but more likely it is the N-oxidation of one of the three nitrogen atoms of quetiapine.

The fragmentation spectrum of imp. B is very similar to the fragmentation spectrum of imp. A. It was suggested that the imp. B is also a sulfoxide with a different location of hydroxyl substituent.

The imp. F, however, showed a different fragmentation spectrum than the impurities A and B. The loss of water molecule was not observed. The imp. F is therefore probably not a regional isomer with hydroxyl group. Moreover, there are no signals other than 48 Da as it was in the case of impurities A, B and E. It was suggested that the impurity might be a sulfone. This hypothesis is supported by the comparison of the peak areas of imp. D and E that shows that the oxidation of the sulfur atom is preferred (peak E is bigger than peak D). The oxidation of the carbon atom in the ring (hydroxyl at the ring) or of the nitrogen atom increases the polarity of the compound (the retention time of imp. D is shorter than for imp. E). The comparison of imp. A (or B) peaks and the peak of imp. E, which is a sulfoxide, proves that the oxidation of sulfoxide in the ring or of the nitrogen atom (e.g., imp. A) results in a significant increase of polarity. The compound F cannot contain two hydroxyl groups at the rings or at the nitrogen atoms; otherwise it would be eluted faster than the imp. E and more likely faster than the imp. A. An ion at m/z 352 is observed on the fragmentation spectrum of the imp. F, which reflects the loss of neutral 64 Da molecule. This molecule might be sulfone (sulfur dioxide). Identification of the main ions, observed on the fragmentation spectra of impurities A, B and F, is presented in Table 3.

The (+) ve electrospray ionization (ESI) spectrum of the C impurity showed peaks at m/z 432 and 454, for protonated and sodium compounds, respectively, which accounts for 48 Da rise compared to quetiapine. On the fragmentation spectrum of the imp. C there are no ions which reflects the loss of neutral 48 Da molecule, i.e., SO. It was concluded that the imp. C might be a sulfone with an addition-

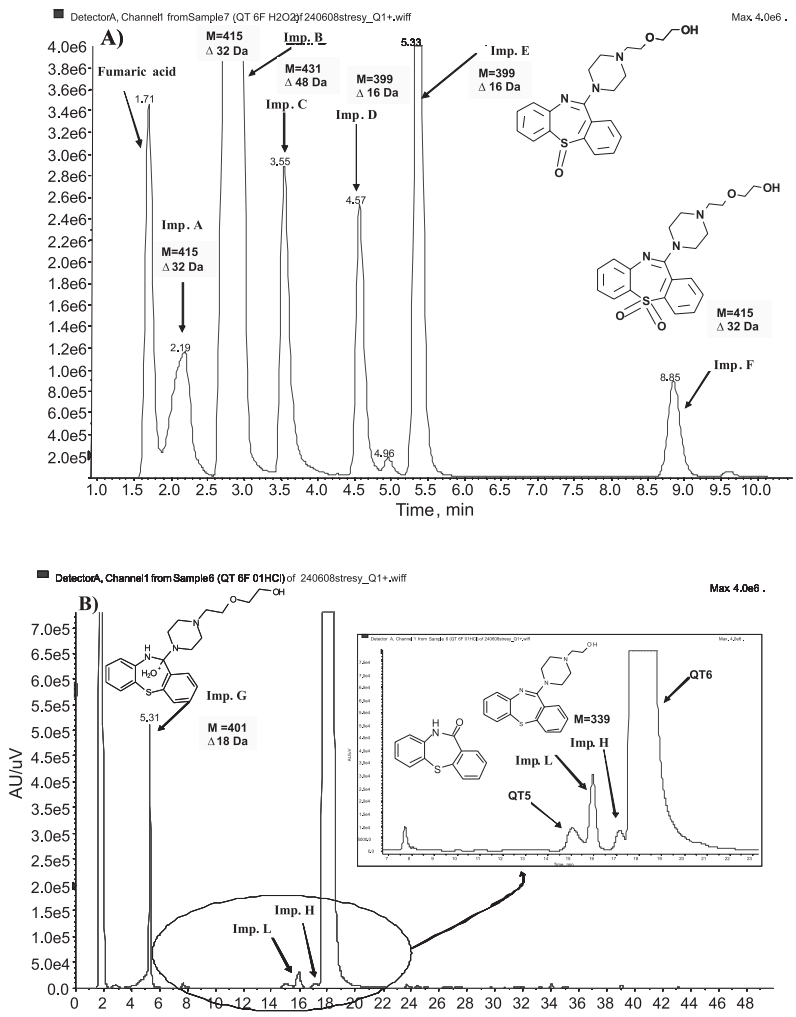
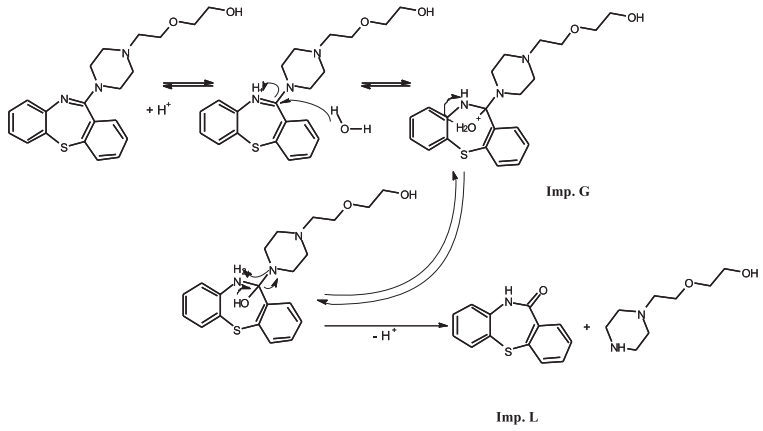


Figure 5. UV chromatograms **A**) Impurity profile of quetiapine in oxidation conditions **B**) Impurity profile of quetiapine in acidic conditions

A

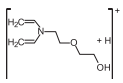
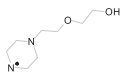
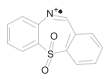
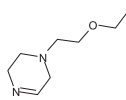


B

$$R_3N + H_2O_2 \longrightarrow R_3N \rightarrow O + H_2O$$

Figure 6. Proposed scheme of quetiapine degradation: **A**) in acidic conditions **B**) in oxidizing conditions

Table 3. Identification of main fragmentation ions of degradation impurities of quetiapine.

Compound (MW, Da)	m/z
Imp. D (399)	356: [M+H – C2H4O]$^+$, 324: [M+H – $C_3H_8O_2$]$^+$, 311:[M+H-$C_4H_9O_2$]$^+$
Imp. E (399)	352: [M+H – SO]$^+$, 296: [M+H – HNC_2H_4O C_2H_4OH], 269: [M+H – C_2H_4N C_2H_4O C_2H_4OH]$^+$, 248: [M+H – HNC_2H_4O C_2H_4OH – SO]$^+$, 221: [M+H – C_2H_4N C_2H_4O C_2H_4OH – SO]$^+$, 158: 
Imp. A (415)/ Imp. B (415)	368: [M+H – SO]$^+$, 35O: [M+H – SO – H_2O]$^+$, 285: [M+H – C_2H_4N C_2H_4O C_2H_4OH]$^+$, 267: [M+H – C_2H_4N C_2H_4O C_2H_4OH – H_2O]$^+$, 247: [M+H – SO – H_2O – HNC_2H_4O C_2H_4OH]$^+$
Imp. F (415)	352: [M+H – *SO2T*, 312: [M+H – HNC_2H_4O C_2H_4OH]$^+$, 286: [M+H – CH_2=CHN C_2H_4O C_2H_4OH]$^+$, 243: [M+H – ]$^+$ 243: 
Imp. C (431)	415: [M+H – OH]$^+$, 388: [M+H – CH_2=CH-OH]$^+$, 327: [M+H – $H_2NC_2H_4O$ C_2H_4OH]$^+$, 312: [M+H – OH – HN=$CHCH_2O$ C_2H_4OH]$^+$, 285: [M+H – CH_2=CH-OH – HN=$CHCH_2O$ C_2H_4OH]$^+$, 283: [M+H – $H_2NC_2H_4O$ C_2H_4OH – CH_2=CH-OH]$^+$ 260 [M+H – ]$^+$
Imp. G (401)	384: [M+H – H_2O] , 340: [M+H – H_2O – CH_2=CHOH]$^+$, 309: [M+H – H_2O – CH_2=CHOH – OCH_3]$^+$, 228: 

al hydroxyl group. The ion at m/z 415 is observed on the fragmentation spectrum of the imp. C, which reflects the loss of an hydroxyl and two ions at m/z 388 and 283 are observed which reflects the loss of neutral 44 Da molecule, which can be the fragment of a substituted ring (CH_2=CH-OH). It was concluded that the imp. C is a sulfone with an additional hydroxyl in the ring. Again, it can not be predicted in which ring and in which position are hydroxyls located. Identification of the main ions, observed on the fragmentation spectrum of impurity C, is presented in Table 3.

Degradation under acidic conditions: Two major peaks are observed on the chromatogram of the sample in acidic conditions (Fig. 5B). The peak observed at RT 16 min (imp. L) is derived from the lactam. This was further confirmed by comparing the retention times of the standard impurity with that of the analyzed peak. The peak observed at RT 17.3 min comes from the imp. H, which has been described in detail in the paragraph: "Identification of the synthesis impurities". The prominent degradation was observed at RT ~ 5.3 min (imp. G).

The (+) νe electrospray ionization (ESI) spectrum of the G impurity showed peaks at m/z 402 and 424, for protonated and sodium compounds, respectively, which accounts for 18 Da rise compared to quetiapine. An m/z 384 ion is observed on the fragmentation spectrum of the imp. G, which reflects the loss of neutral 18 Da molecule – probably H_2O. Therefore, it was assumed that the imp. G was formed through the addition of water molecule to the quetiapine molecule. The fragmentation of the imp. G is different from the fragmentation of quetiapine. The fragmentation of ethoxyethanol chain is observed in the impurity G. Therefore, the piperazine system is more stable than the one of quetiapine. In addition, ion at m/z 228 is important, which corresponds to the lactam structure. These data suggest the addition of water molecule to the carbon atom of substituted piperazinyl group. The identification of the main ions observed on the fragmentation spectrum of the imp. G is presented in Table 3.

CONCLUSIONS

The main aim of this work was to develop the method for the impurity profile determination in quetiapine as an API using chromatography and mass spectrometry. The synthesis of quetiapine developed at Pharmaceutical Research Institute is new and it is a subject of patent application (17). Therefore, the impurity profile of this substance is also new.

It was shown that liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is a suitable tool for the identification of organic impurities in the pharmaceutical substance of interest. Using these techniques, the methods for separation and identification were developed which allow to determine both, process impurities and degradation impurities (which were formed during the stability study of the tested substance). The methods of the impurity profile determination fulfill the initial criteria: they give the possibility of following a wide range of impurities in the optimum time of the analysis, they are sensitive and selective. The methods of the impurity profile determination allow to monitor the changes in the impurity profile, resulting from both intended and unintended changes during the synthesis or during the storage of API. The methods also enable to confirm identity of the API of study and structure elucidation and identification of the impurities from the synthesis as well as the impurities resulting from the degradation, which could not be obtained as standards. The mass spectra, fragmentation spectra and chromatographic data were used to identify the impurities. The proposed structures of process impurity were additionally confirmed by analysing the route of synthesis of the substance.

Difficulties in the analysis of degradation impurities of quetiapine, using the MS technique, mainly resulted from the fragmentation of the side chain, which led to the formation of unrepresentative fragments. Despite these difficulties, some interesting conclusions were drawn. Most of the degradation products were observed in 3% hydrogen peroxide. In these conditions the main degradation products were successfully identified. Quetiapine undergoes a single, double and triple oxidation. The experiments showed that the sulfur atom is oxidized first, followed by oxidation of the ring or nitrogen atom of quetiapine. The oxidation of the sulfur atom is preferred, as demonstrated by comparing the peak of the imp. E, which is larger than the peak of the imp. D. On the basis of the MS spectra only, it is not possible to predict the location (nitrogen atoms, rings) where the oxidation occurred. The presence of subsequent oxygenated groups increases compound polarity, which affects the retention time. The compounds from A to D were eluted faster than the imp. E, considered to be sulfoxide. Under the conditions of acidic hydrolysis, two major degradation products are observed. The main degradation impurity is a compound created by the addition of the water molecule to quetiapine molecule. The fragmentation spectrum of this impurity, in particular

the characteristic ions, indicate the stabilization of the lactam ring. This suggests that water molecule was linked to the carbon atom substituted with a piperazine group.

Stress study of quetiapine allowed to define the initial conditions of synthesis and critical steps in the synthesis of quetiapine. Information on degradation products structures leads to the development of new compounds with improved stability and facilitates the design of more stable formulations.

The information resulting from this study allowed to identify the impurity degradation G, which appeared also in quetiapine samples during the upscaling of the synthesis. Figure 6 suggests quetiapine degradation mechanisms on the basis of identified degradation impurities.

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REFERENCES

1. Kinon B.J., Noordsy D.L., Liu-Seifert H., Gulliver A.H., Ascher-Svanum H., Kollack-Walker S.: J. Clin. Psychopharmacol. 26, 453 (2006).
2. Onrust S.V., McClellan K.: CNS Drugs 15, 329 (2001).
3. Andrezina R., Josiassen R.C., Marcus R.N., Oren D.A., Manos G., Stock E., Carson W.H., Iwamoto T.: Psychopharmacology (Berl) 188, 281 (2006).
4. Nandkumar Ch.: Impurities in drug substance & products. USP 6th Annual Scientific Meeting, India. www.usp.org/pdf/EN/eventsEducation/asMeeting/2007India/session1Track1_01Chondankar.pdf.
5. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Q3A (R2): Impurities in new drug substances, October 2006, http://www.ich.org/LOB/media/MEDIA422.pdf.
6. Gavin P.F., Olsen B.A., Wirth D.D., Lorenz K.T.: J. Pharm. Biomed. Anal. 41, 1251 (2006).
7. Lewen N., Mathew S., Schenkenberger M., Raglione T.: J. Pharm. Biomed. Anal. 35, 739 (2004).
8. Otero R., Carrera G., Dulsat J.F., Fábregas J.L., Claramunt J.: J. Chromatogr. A 1057, 193 (2004).
9. Sheldon E.M., Downar J.B.: J. Pharm. Biomed. Anal. 23, 561 (2000).
10. Argentine M.D., Owens P.K., Olsen B.A.: Adv. Drug Deliv. Rev. 59, 12 (2007).
11. International Conference on Harmonisation (ICH) Guidelines, Q3B(R): Impurities in New Drug Products (Revised Guideline), February 2003.
12. Harmonised Tripartite Guideline on Impurities: Residual Solvents (Q3C), International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), Geneva 1997.
13. Görög S.: J. Pharm. Biomed. Anal. 36, 931 (2005).
14. Smith R.J., Webb M.L.: Analysis of drug impurities, Blackwell Publishing, Oxford 2007.
15. Lim Ch.-K., Lord G.: Biol. Pharm. Bull. 25, 547 (2002).
16. Stolarczyk E.U., Kaczmarek Ł., Eksanow K., Kubiszewski M., Glice M., Kutner A.: Pharm. Dev. Technol. 14, 27 (2009).
17. Kaczmarek Ł., Badowska-Rosłonek K., Stolarczyk E., Szelejewski W.: Process for preparation of 11-(1-piperazinyl)dibenzo[b,f][1,4]thiazepine, an intermediate in the synthesis of the antipsychotic drug quetiapine, PCT/EP2004/051520, 2004.