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LC-MS and CE-MS Strategies in Impurity Profiling

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Abstract. LC-MS has become an important tool in all areas of drug discovery and development within pharmaceutical industry. It is very suitable when searching for impurities in drugs, such as reaction byproducts or degradation products. Various strategies for the identification of drug impurities are critically evaluated. In addition, the potential of CE-MS in impurity profiling is discussed.

Introduction

In the past few years, the application of LC-MS has grown spectacularly [1]. Especially within pharmaceutical industries, the use of LC-MS is currently involved in all steps of the drug-development process. In drug discovery *via* either intelligent synthesis or combinatorial chemical approaches, LC-MS is used for the rapid determination of molecular masses of the reaction products prior to biological screening. In addition, LC-MS-controlled fractionation and preparative liquid chromatography is of growing importance in this field. At a later stage in the drug development, LC-MS, often in combination with other techniques like NMR, plays a key role in identification of lead compounds, their impurities, degradation products as well as their metabolites. The most important application in the pharmaceutical field is the use of LC-MS and especially LC-MS-MS in selective reaction-monitoring mode (SRM) in quantitative bioanalysis.

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The present contribution focuses on the applications of LC-MS and related techniques in impurity profiling and degradation studies. For proprietary reasons, little information is available on this type of applications in the public literature. General strategies in impurity profiling as applied and applicable within pharmaceutical industries are reviewed and critically evaluated and, where possible, illustrated with examples from literature.

LC-MS Technology

In the past ten years, the field of LC-MS has changed significantly [1]. While in the past a great variety of LC-MS technologies, *e.g.*, based on particle-beam, thermospray and continuous-flow fast-atom bombardment interfaces, had to be applied in order to solve analytical problems, at present, LC-MS is performed with only two interfaces, namely electrospray (ESI) and atmospheric-pressure chemical ionization (APCI), which generally share most of the instrument hardware, thus enabling easy changeover from ESI to APCI and *vice versa*. In terms of ionization, APCI and ESI share many common features, especially for the majority of the compounds of pharmaceutical interest, *i.e.*, generally polar compounds with molecular masses up to typically 1000 Da. With both ionization techniques, predominantly protonated molecules are generated in positive-ion mode and deprotonated molecules in negative-ion mode. In addition, a number of adduct ions may be observed. In general, ESI is more prone to adduct formation, especially to the generation of sodiated molecules. Both ESI and APCI provide soft ionization. Generally, no fragmentation is observed, unless it is collisionally induced, either in the ion source by increasing the potential difference between the sampling cone or capillary exit (in-source CID) and the skimmer, or *via* tandem mass spectrometry (MS-MS) in triple-quadrupole, ion-trap or other (hybrid) systems.

General Aspects of Impurity Profiling

Limits for the presence of impurities in active pharmaceuticals are established by the International Conference on Harmonization (ICH), which is active in the coordination of technical requirements for the registration of pharmaceuticals in the United States, Japan, and the European Union [2–4]. All drugs contain impurities, resulting from many sources, *e.g.*, raw materials and reagents, reaction byproducts, and due to degradation during manufacture and storage. Three types of impurities must be distinguished: inorganic and organic compounds, and solvent residues. In this paper, especially the organic impurities are of interest.

ICH Regulations are applicable to new synthetic chemical entities, which were not previously registered. The regulatory bodies demand qualification of the impurities present in drugs and their formulations down to the 0.1% *w/w* level or 1 mg/day, whichever is lower (for drugs administered at up to 2 g per day). The qualification required is defined as the process of acquiring and evaluating data which es-

tablish the biological safety of the individual impurity or a given impurity profile at the level(s) specified, *i.e.*, efforts from both analysis and toxicology are needed.

In addition to regulatory affairs, another purpose of impurity profiling can be patent protection of, *e.g.*, a particular synthesis for a compound. In the latter case, synthetic-route-indicative impurities are searched for in competitors' products, often at much lower levels than 0.1%.

In practice, the 0.1% level is often based on UV detection after LC separation, assuming identical molar absorptivity of the major compound and its impurities. This implies that a limit of quantification of *ca.* 0.05% is required for the impurities [3].

Impurity profiling always deals with the detection and identification of minor components next to a major component, *i.e.*, the drug itself. In many separation/detection systems, this may lead to experimental difficulties related to, *e.g.*, column overloading, limited linearity and/or dynamic range of the detection system, and the different detection characteristics of the impurities.

Some of these problems are clearly present in the application of LC-MS in the impurity-profiling studies. ESI is known for its limited dynamic range [5][6]. However, it is obviously not necessary to detect both the major component and the minor impurities in a single run, enabling ways to circumvent the limited dynamic range of the LC-ESI-MS. In some instances, even special precautions must be taken, *e.g.*, by diverting away the major component from the ion source by valve-switching techniques, in order to avoid source contamination and/or especially ion suppression when analysing the minor impurities.

Given the large differences in response factors between structural analogues, as frequently observed in ESI, the major role of LC-MS is not in the quantitative assessment of impurity, but more in the qualitative analysis, *i.e.*, structure elucidation and identification, of the impurities present. Due to the significantly different detection characteristics, compounds that show up in the chromatogram from the UV photodiode-array detection (UV-PDA) may turn out to be 'transparent' in LC-MS detection and *vice versa*.

A major problem in this type of studies is the general assessment of chromatographic peak purity. Elaborate research on peak-purity assessment has been performed for UV-PDA detection. This resulted in a number of univariate approaches, *e.g.*, spectral overlays, iso-absorbance plots, derivative transformations, and numerical approaches, *e.g.*, absorbance ratioing, purity parameters, and multiple absorbance-ratio correlation. More powerful methods in peak-purity assessment are based on multivariate analysis, *e.g.*, principal-component analysis and factor analysis [7][8]. The latter have also been applied to data from LC-MS [7][9][10].

Another topic related to data processing results from the fact that in LC-MS, the analyte peaks have to be identified against a relatively high background of solvent-related ion current. While a total-ion chromatogram (TIC) in GC-MS often reveals the presence of a number of compounds, even at low injected amounts, this is not necessarily true in LC-MS. The search for unknown compounds at low concentrations is often difficult in LC-MS. Generation of reconstructed mass chromatograms enables the detection of these hidden peaks, but searching for peaks over a wide mass range might be a tedious and time-consuming procedure. The use of base-peak chromatograms can be helpful in this respect, but the implementation of the base-peak chromatograms in most commercial MS-software packages is rather poor: in most cases, the *m/z* range to be searched for base peaks cannot be specified. More powerful chemometric approaches can assist in peak recognition in a TIC trace [9][10]. However, efficient tools for peak-purity assessment, such as available in, for instance, the *Waters Millenium 32* chromatography software [11], are absent in most commercial MS-software packages. An univariate peak-purity assessment based on the degree of overlap of normalized spectra at the upslope, apex, and downslope of the chromatographic peak is available in the software delivered with the *Hewlett-Packard HP-1000* LC-MSD system [12]. To our knowledge, applications of this tool in impurity profiling have not been reported yet.

Detection and Identification of Impurities by LC-MS

A general, conventional approach towards the detection and identification of drug impurities and degradation products implies a number of steps: LC separation of sample batches using UV-PDA or multiple detection strategies and a wide solvent-gradient programme, synthesis of expected impurities, *e.g.*, based on knowledge of the synthetic route and the chemistry involved and/or predicted by computer-aided molecular modeling (CAMM). Fractionation and/or preparative LC are further steps to obtain sufficient amounts of the impurities for identification by MS, nuclear magnetic resonance spectroscopy (NMR), and other techniques. Several examples of this strategy are available from the literature.

An early account on the application of LC-MS in impurity analysis is the confirmation of the structure of three synthesis byproducts of the awakening drug modafinil ((diphenylmethyl)sulfinyl-2-acetamide, 273 Da) by means of LC-thermospray-MS, as reported by *Becue* and *Broquaire* [13]. Repeller-induced in-source CID was applied to induce fragmentation. The mass spectra of three impurities obtained this way, contained sufficient structure-informative fragmentation to enable comparison with mass spectra of standards obtained under identical conditions.

A LC-thermospray-MS method for the detection of the three synthetic-route-indicative impurities of 3'-azido-3'-deoxythymidine (AZT, 267 Da) at 50–100 ppb levels was described by *Almudaris et al.* [14] for the purpose of patent protection. The bulk powder or the pharmaceutical product was extracted and dissolved in an acetonitrile/1,2-dichloroethane mixture. The extract was fractionated by normal-phase chromatography. The fractions were subsequently analysed by reversed-phase LC-thermospray-MS to enable sensitive detection of the impurities, eluting just in front of the parent compound.

A low-level degradation product was found during a package-screening study in film-coated tablets of the H2-receptor antagonist famotidine (3-[({2-[(amino-iminomethyl)amino]thiazol-4-yl}methyl)thio]-*N*-(aminosulfonyl)propanimidamide, 337 Da) [15]. Using LC-APCI-MS in the positive-ion mode, the product was found to have a molecular mass of 349 Da, corresponding to the addition of a carbon atom. Using LC-MS-MS, it was found that the carbon was added to the side of the *N*-(aminosulfonyl)propanimidamide moiety of famotidine. The tentative structure, proposed from the MS-MS product-ion mass spectrum, was confirmed by means of synthesis and subsequent analysis of the synthetic product.

In one of the peak-purity assessment methods, UV or mass spectra obtained at various points across the chromatographic peak are compared to the spectrum obtained at the apex of the peak. This approach assumes that the main component is purest at the apex and requires that the spectra of the impurities are slightly different from the spectrum of the major component. In UV detection, the latter

condition may not be met for impurities closely related to the major component. In MS detection, a difference in *m/z* between the impurities and the major component is required and sufficient.

Bryant et al. [16] investigated the potential of ESI-MS in purity assessment of (deliberately) coeluting peaks of a major component and a number of related impurities. As they essentially pursued the detection of the impurities based on differences in molecular mass, the setting of the voltage at the sampling cone or capillary exit had to be carefully optimised. At low voltages, the positive-ion mass spectrum contains intact cationised analyte species, together with a number of solvent-cluster ions. At higher voltages, the latter disappear, but fragment ions may appear in addition to intact cationised species. At still higher voltages, the fragment ions become increasingly more intense. Intermediate voltages were applied by *Bryant et al.* [16]. In this way, coeluting impurities of famciclovir and ropinirole spiked at a 0.1% level, could be detected. The impurities studied have UV spectra that are very similar to the major component. Due to the differences in response factors between even closely related components, quantitative data are unreliable: a ropinirole-related impurity spiked at the 0.1% level shows a 0.7% relative abundance in the mass spectrum of ropinirole.

Detection and Identification of Impurities by LC-MS-MS

In many studies on impurities and degradation products, various MS strategies are applied, or LC-MS is performed on an MS-MS instrument.

Three unknown products were observed in the LC analysis of severely stressed losartan (the potassium salt of 2-butyl-4-chloro-5-hydroxymethyl-1-{[2'-(1*H*-tetrazol-5-yl)biphenyl-5-yl]methyl} imidazole, 423 Da) tablets [17]. APCI LC-MS-MS was applied to identify these three products as an aldehyde and two dimeric derivatives of losartan, which are present at sub-0.1% levels. The structural assignment was further confirmed by comparing the MS-MS spectra of the degradation products with those of the synthesized products.

Multiple MS-ionization techniques, *i.e.*, electron ionization, fast-atom bombardment, and electrospray, were applied by *Raffaelli et al.* [18] in the identification of a synthesis by-product of pyrazolotriazolopyrimidines. The impurity was due to an ethyl substitution at one of the rings. The position was established with ^{1}H-NMR.

An important step in the identification procedure of impurities and degradation products is the use of MS-MS in product-ion scan mode. Fragmentation of protonated molecules in low-energy CID, *i.e.*, in triple-quadrupole or ion-trap instruments, often leads to a limited number of fragments. In those cases, unambiguous identification of the impurity will be difficult. In addition, the interpretation of the MS-MS spectra is often hampered by the lack of knowledge of the fragmentation rules. Unlike the fragmentation of the odd-electron radical cations generated in electron ionization, little systematic knowledge is available on the mechanisms of the fragmentation of even-electron protonated molecules. However, in case of the pyrazolotriazolopyrimidines [18], the fragmentation in the MS-MS spectra based on both protonated and deprotonated molecules was found to be far more informative than that in the electron-ionization spectrum.

LC-ESI-MS(-MS) was applied to identify a number of degradation products from the bulk drug form of calcium-channel blocker isradipine (isopropylmethyl 4-(benzofurazan-4-yl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate, 371 Da) by *Bartlett et al.* [19]. Surprisingly, the most abundant ion in the mass spectrum of the parent drug is the sodiated molecule, while for its degradation products, the protonated molecule is most dominant. This is surprising for at least one of the degradation products, in which a methyl ester in the parent drug is converted into a carboxylic-acid function, which is expected to be more prone to the formation of sodiated molecules. In this particular case, MS-MS is of little use, because the major fragment ions correspond to losses of the two ester moieties (methyl and i-propyl) without much further fragmentation of the skeleton.

MS-MS is not only an important tool for identification purposes by means of the product-ion scan mode, but also for screening for impurities and degradation products by means of the precursor-ion mode and/or the neutral-loss scan mode. The screening in both modes is based on common structural features, *i.e.*, common product ions or common neutral losses [20][21].

The application of these MS-MS screening strategies in impurity profiling was demonstrated by *Volk et al.* [22] for butorphanol tartrate, an intranasal analgesic applied in the treatment of post-surgical pain. The product-ion mass spectrum of protonated butorphanol contains a series of structure-informative fragments. Based on the premise that targeted degradation products will retain much of the original butorphanol structure, and therefore undergo similar fragmentation, precursor-ion scans, *e.g.*, using *m/z* 199 due to the three-membered butorphanol ring core and subsequent fragmentation to *m/z* 171, 157, and 145, as well as neutral-loss scans, *e.g.*, loss of ethene (28 Da), cyclobutane (58 Da), can be performed to screen for the degradation products. In this way, five degradation products in long-term stability studies could be identified.

Screening for erythromycin A degradation products by means of precursor-ion scan was described by *Volmer* and *Hui* [23]. In this study, the degradation of erythromycin A in aqueous solutions in the pH range 2–13 was studied. Extraction of the degradation products from the solution was achieved by means of solid-phase microextraction (SPME). The SPME fiber was subsequently inserted into the desorption chamber of the SPME–LC interface. After elution of the SPME fiber, a fast gradient was applied to a 50-mm column to achieve an analysis time of *ca.* 10 min. Next to the major degradation product, anhydroerythromycine A and a variety of other products were detected and tentatively identified from the MS-MS product-ion mass spectrum.

Overcoming Limitations of LC-MS-MS

As indicated, the interpretation of the MS-MS product-ion mass spectra is not always straightforward. The fragmentation of even-electron ions is characterized by frequent rearrangements, especially hydrogen-shifts. Various tools have been developed to simplify structure elucidation. Most of these tools were developed in order to facilitate the interpretation during metabolic studies, but they should be similarly applicable to assist in mass-spectral interpretation problems related to impurity and degradation studies. H/D Exchange was demonstrated by *Siegel* [24] for the LC-thermospray-MS analysis of tetracyclins, β-lactam antibiotics, peptides, and various other compounds, and by *Karlsson* [25] and by *Gard et al.* [26] in the LC-ESI-MS analysis of peptides. A recent application in drug-metabolism studies was described by *Ohashi et al.* [27].

As indicated above, *e.g.*, in the study on isradipine [19], the structure information obtained in product-ion MS-MS spectra is sometimes limited. In some cases,

the combination of in-source CID, in order to generate a relatively stable fragment due to the cleavage of a weak bond, and product-ion MS-MS, in order to fragment the ions generated in in-source CID, can be helpful. Clear examples of this approach were reported by *Chaudhary et al.* [28] for an adduct between guanosine and malondialdehyde, and by *Bateman et al.* [29] for the structure elucidation of isomeric sulfonamides.

The often softer fragmentation during CID in ion-trap instruments can also be of great help for identification and structure-elucidation purposes. Multiple stages of product-ion MS-MS are available for these instruments, thus allowing a stepwise fragmentation of the ions of interest. Some examples of this approach are available in metabolism studies, *e.g.*, the elucidation of the glyburide metabolism, as described by *Tiller et al.* [30], and the identification of urinary metabolites of a novel non-nucleoside reverse-transcriptase inhibitor (GW420867), as described by *Dear et al.* [31].

Another tool for structure elucidation is the application of high-resolution MS, allowing the determination of accurate masses, which in turn enables calculation and/or confirmation of elemental compositions. An early example of this approach is the application of high-resolution MS on a sector instrument and B/E linked scanning in the characterization of synthesis byproducts of the HIV-1 reverse-transcriptase inhibitor BI-RG-587 using thermospray LC-MS [32]. Seven byproducts were detected, some differing in the position of the cyclopropyl group and some having a different side chain as well as one bearing a chlorine substituent.

Another approach is high-resolution MS by means of *Fourier*-transform ion-cyclotron resonance MS, *i.e.*, *via* on-line LC-electrospray-FT-ICR-MS, as demonstrated by *Haskins et al.* [33]. The LC chromatogram of four reaction byproducts sometimes observed in cimetidine shows a poor separation, but accurate mass measurement (at 8–9 ppm) confirmed the identity of the components. Furthermore, a number of additional process impurities were detected. Some could be identified from accurate mass determination.

Recently, a very powerful alternative in structure elucidation by means of MS-MS was introduced: a hybrid instrument consisting of a front-end quadrupole and a back-end orthogonal acceleration time-of-flight analyser [34]. This type of hybrid instrument, the so-called Q-TOF, yields high full-scan sensitivity because of the excellent duty cycle of the time-of-flight analyser and high-resolution mass determination at 5 ppm with internal mass calibration using a single-lock mass, *e.g.*, by means of the precursor ion. This system was evaluated for impurity and degradation studies of cimetidine by *Eckers et al.* [35] and *Lee et al.* [36]. In the latter study, the high-resolution capabilities of the Q-TOF instrument were tested in combination with fast-gradient LC, *i.e.*, typical run times of 5 min and narrow chromatographic peaks. Data were shown for degradation products of cimetidine. With one exception (12.5 ppm), the accurate masses of six products could be determined experimentally with a error less than 3.1 ppm. With this accuracy, the elemental composition of the degradation product can be calculated with high confidence.

LC-NMR in Impurity Studies

As indicated above, NMR is an important tool in studies related to impurities and degradation products of pharmaceuticals. Often, fraction collection after preparative LC is required to successfully perform NMR. A recent example of this approach, where off-line NMR is applied in conjunction with LC-MS, was reported by *Lehr et al.* [4]. Two impurities were found in various batches of trimethoprim (2,4-diamino-5-(3,4,5-trimethoxybenzyl)-pyrimidine, 290 Da), obtained from five different manufacturers in three different countries. These impurities were not detected by the compendial method, although they were present at significant levels. After isolation by means of preparative LC, the two compounds were identified using LC–APCI–MS–MS and NMR as 2,4-diamino-5-(4-ethoxy-3,5-dimethoxybenzyl)pyrimidine and 2,4-diamino-5-(3-bromo-4,5-dimethoxybenzyl)pyrimidine.

Significant progress has been made in the on-line combination between LC and NMR, avoiding the need for fraction collection prior to NMR analysis [37]. Given the importance of NMR data in structure elucidation of impurities and degradation products, *e.g.*, in the elucidation of regional isomerism, the use of on-line LC-NMR in such studies is a promising development.

Peng et al. [38] recently described the use of LC-NMR next to LC-MS for the identification of six degradation products of a protease inhibitor, *N*-hydroxy-1,3-i-[4-ethoxybenzenesulfonyl]-5,5-dimethyl-[1,3]cyclohexyldiazine-2-carboxamide, in a dosage formulation. In LC-MS studies, ESI was used with alternating scans at low and high cone voltages, in order to induce fragmentation by in-source CID. The LC-MS data led to tentative structure assignments. For three of the six degradation products, unambiguous identification appeared not to be possible. Two structures were proposed for one product, while three structures were proposed for the two other isomeric degradation products (452 Da). Subsequently, LC-NMR was applied to confirm the structures of the products for which only one tentative structure was proposed as well as to select the correct structure from the isomeric or isobaric structures proposed for the other three products. Good-quality NMR spectra were obtained using 5–50 μg of each component injected on-column. LC-NMR was performed under conditions identical to the LC-MS studies.

On-line LC-NMR is still at its infancy: interesting developments are to be expected, which will strengthen its applicability in structure-elucidation studies.

Impurities in Combinatorial Libraries

Impurity assessment is also an important aspect in the characterization of compound libraries obtained by high-throughput parallel synthesis. Next to rapid screening of libraries by column-bypass MS or fast LC-MS [39][40], purity and/or other quality-control issues are becoming increasingly important [4–43]. A rapid method for this purpose, based on reversed-phase LC separation on 50-mm long columns and on-line UV-PDA and APCI-MS detection, was described by *Duléry et al.* [42]. Selected compounds are subsequently subjected to off-line NMR analysis. Purity assessment is based on UV-PDA data, while NMR and MS are applied for compound identification.

A combination of methods, *i.e.*, LC coupled on-line to nitrogen-sensitive chemiluminescent, UV, and MS detection, was proposed for the characterization of small compounds derived from combinatorial libraries [43]. The method enables simultaneous determination of identity, quantity, and purity of the product analysed.

This is an area where significant developments can be anticipated in the near future.

Impurity Detection in Peptides by LC-MS

Synthetic peptides are becoming increasingly important as drugs in the phar-

maceutical industry. The synthesis is mostly performed by stepwise solid-phase synthesis. This procedure often results in unwanted side products associated with incomplete peptide chains. A number of papers are devoted to studies on impurities and peptide degradation (see, *e.g.*, [44] and refs. cited therein). The selection below is certainly not comprehensive, but is meant to indicate some of the problems associated with this type of analysis.

In an early study, the products from the solid-phase synthesis of the acyl-carrier protein ACP(65–74) decapeptide (VQAAIDYING) were investigated by *Schnölzer et al.* [45]. The components were separated and fractionated by HPLC. The fractions were subsequently analysed by means of ESI-MS-MS. A major by-product was arising from incomplete deprotection of Asp70. In addition, trace by-products were found to arise from succinimide formation at Asp70 or Asn73, acylation of the Tyr71 side-chain hydroxygroup, leading to a branched heptadecapeptide, and *tert*-butylation of the decapeptide.

The composition and purity of synthetic peptide mixtures, containing 48 peptides in equimolar amounts, was evaluated by *Metzger et al.* [46]. The main by-products were due to incomplete removal of side-chain-protecting groups. Precursor-ion and neutral-loss scan modes were applied for the rapid detection of peptides modified with the same protecting group.

An important topic in impurity profiling is the chirality of drugs. However, in the discrimination between enantiomers, MS can play only a minor role. A method for determination of the optical purity of amino acids in small peptides was reported by *Goodlett et al.* [47]. The peptides of interest were hydrolysed in deuterated acids, enabling the MS detection of racemization during the acid hydrolysis. The resulting amino acids were converted to diastereoisomers by derivatization with *Marfey*'s reagent (*N*-(5-fluoro-2,4-dinitrophenyl)-L-valinamide). Mixtures of diastereoisomers were separated by reversed-phase LC and detected by means of ESI-MS. The method was applied to the determination of the chiral purity of a hexapeptide by-product (RKKDVY) in batches of the synthetic pentapeptide thymopentin (RKDVY).

Sanz-Nebot et al. [48] recently reported the use of LC-ESI-MS to identify solid-phase-synthetic byproducts in the preparation of the peptide drug carbocetin. For some impurities, structure-informative fragmentation could be obtained by means of in-source CID.

The Role of CE-MS in Impurity Profiling

One of the requirements of the separation techniques applied in impurity and degradation studies is a high separation power for the compounds of interest. Both selectivity and efficiency of the column are of utmost importance. Capillary electromigration techniques such as capillary zone electrophoresis (CZE) show a very high performance for both. Therefore, CZE would be a powerful alternative to LC for a number of separation problems. In combination with UV detection, CZE has been successfully applied in impurity profiling of pharmaceuticals. Despite the great promises of on-line CZE-MS [49], the practical application of CZE-MS is still limited. This may be due to some inherent sensitivity problems related to CE-MS [1].

The potential of CZE-MS in the detection of impurities in peptides and alkaloids was investigated by *Hsieh et al.* [50]. Met-Lys-bradykinin was deliberately contaminated with 0.1% Lys-Bradykinin. It was found that CZE-MS in selected-ion monitoring mode (SIM) was capable of detecting the impurity at the 0.1% level, while CZE-UV was not. Both CZE-UV and CZE-MS in SIM mode could detect the presence of 0.15% palmitine in the alkaloid berberine. The SIM or SRM mode is a powerful tool in the low-level detection of known impurities, *e.g.*, in quality-control studies. However, it does not allow the detection of unknown impurities.

A more real-life application of CZE-MS in this area was reported by *Hoitink et al.* [51]. CZE-MS was investigated as a tool in the stability research of the luteinising-hormone-releasing hormone analogue goserelin. In CZE, the peptide-like degradation products elute within a relatively small time window, enabling a shorter analysis time than in LC. However, unlike in reversed-phase LC, the diastereoisomers are not separated by CZE. Unfortunately, no clear indications are given on the concentration levels of the degradation products analysed compared to the parent compound. With the advent of dynamic nanoelectrospray techniques, enabling direct coupling of the CZE column to the highly efficient nanoelectrospray source, further growth and developments in this field might be expected.

Conclusion

LC-MS can be a powerful tool in studies related to the detection and identification of impurities and degradation products of pharmaceuticals. Its main application area is structure elucidation rather than quantitative purity assessment. In most cases, LC-MS, even in combination with various MS-MS strategies, will not provide an unambiguous compound identification. Other spectroscopic techniques, especially NMR, as well as confirmative synthesis, must be applied. The recent advent of on-line LC-NMR, and even on-line LC-NMR-MS [52] provides new, powerful tools in impurity profiling. Despite the great promises, the role of CZE-MS in this area is still very limited, due to the inherent sensitivity problems of this technique.

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[1] W.M.A. Niessen, 'Liquid Chromatography – Mass Spectrometry', 2nd Ed., Marcel Dekker Inc., New York, NY., 1999.
[2] International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, 1995, ICH Steering Committee, March 30, 1995.
[3] J.C. Berridge, *J. Pharm. Biomed. Anal.* **1995**, *14*, 7.
[4] G.J. Lehr, T.L. Barry, G. Petzinger, G.M. Hanna, S.W. Zito, *J. Pharm. Biomed. Anal.* **1999**, *19*, 373.
[5] L. Tang, P. Kebarle, *Anal. Chem.* **1999**, *63*, 2709.
[6] A.P. Bruins, *J. Chromatogr. A* **1998**, *794*, 345.
[7] D. Lincoln, A.F. Fell, N.H. Anderson, D. England, *J. Pharm. Biomed. Anal.* **1992**, *10*, 837.
[8] K. de Braekeleer, A. de Juan, D.L. Massart, *J. Chromatogr. A* **1999**, *832*, 67.
[9] W. Windig, J. Phalp, A.W. Payne, *Anal. Chem.* **1996**, *68*, 3602.
[10] E.R. Verheij, Presented at the 15th Montreux Symposium of LC–MS, November 11–13, 1998, Montreux, Switzerland.
[11] *Waters*, Milford. MA, http://www.waters.com.
[12] *Hewlett-Packard*, Palo Alto, CA, http://chem.external.hp.com/cag.
[13] T. Becue, M. Broquaire, *J. Chromatogr.* **1991**, *557*, 489.
[14] A. Almudaris, D.S. Ashton, A. Ray, K. Valko, *J. Chromatogr. A* **1995**, *689*, 31.
[15] X.-Z. Qin, D.P. Ip, K.H.-C. Chang, P.M. Dradransky, M.A. Brooks, T. Sakuma, *J. Pharm. Biomed. Anal.* **1994**, *12*, 221.
[16] D.K. Bryant, M.D. Kingswood, A. Belenguer, *J. Chromatogr. A* **1996**, *721*, 41.
[17] Z. Zhao, Q. Wang, E.W. Tsai, X.-Z. Qin, D. Ip, *J. Pharm. Biomed. Anal.* **1999**, *20*, 129.

[18] A. Raffaelli, S. Pucci, G. Uccello-Barretta, F. Russo, S. Guccione, *Rapid Commun. Mass Spectrom.* **1996**, *10*, 1939.
[19] M.G. Bartlett, J.C. Spell, P.S. Mathis, M.F.A. Elgany, B.E. El Zeany, M.A. Elkawy, J.T. Stewart, *J. Pharm. Biomed. Anal.* **1995**, *18*, 335.
[20] D.F. Hunt, J. Shabanowitz, T.M. Harvey, M.L. Coates, *J. Chromatogr.* **1983**, *271*, 93.
[21] J.V. Johnson, R.A. Yost, *Anal. Chem.* **1985**, *57*, 758A.
[22] K.J. Volk, S.E. Klohr, R.A. Rourick, E.H. Kerns, M.S. Lee, *J. Pharm. Biomed. Anal.* **1996**, *14*, 1663.
[23] D.A. Volmer, J.P.M. Hui, *Rapid Commun. Mass Spectrom.* **1998**, *12*, 123.
[24] M.M. Siegel, *Anal. Chem.* **1998**, *60*, 2090.
[25] K.E. Karlsson, *J. Chromatogr.* **1993**, *647*, 31.
[26] E. Gard, M.K. Green, J. Bregar, C.B. Lebrilla, *J. Am. Soc. Mass Spectrom.* **1994**, *5*, 623.
[27] N. Ohashi, S. Furuuchi, M. Yoshikawa, *J. Pharm. Biomed. Anal.* **1998**, *18*, 325.
[28] A.K. Chaudhary, M. Nokubo, T.D. Oglesby, L.J. Marnett, I.A. Blair, *J. Mass Spectrom.* **1996**, *30*, 1157.
[29] K.P. Bateman, S.J. Locke, D.A. Volmer, *J. Mass Spectrom.* **1997**, *32*, 297.
[30] P.R. Tiller, A.P. Land, I. Jardine, D.M. Murphy, R. Sozio, A. Ayrton, W.H. Schaefer, *J. Chromatogr.* **1998**, *794*, 15.
[31] G.J. Dear, J. Ayrton, R. Plumb, B.C. Sweatman, I.M. Ismail, I.J. Fraser, P.J. Mutch, *Rapid Commun. Mass Spectrom.* **1998**, *12*, 2023.
[32] R.M. Dinallo, W.C. Davidson, G.E. Hansen, *Biol. Mass Spectrom.* **1991**, *20*, 268.
[33] N.J. Haskins, C. Eckers, A.J. Organ, M.F. Dunk, B.E. Winder, *Rapid Commun. Mass Spectrom.* **1995**, *9*, 1027.
[34] H.R. Morris, T. Paxton, A. Dell, J. Langhorne, M. Berg, R.S. Bordoli, J. Hoyes, R.H. Bateman, *Rapid Commun. Mass Spectrom.* **1996**, *10*, 889.
[35] C. Eckers, N.J. Haskins, J. Langridge, *Rapid Commun. Mass Spectrom.* **1997**, *11*, 1916.
[36] M.J. Lee, S. Monté, J. Sanderson, N.J. Haskins, *Rapid Commun. Mass Spectrom.* **1999**, *13*, 216.
[37] K. Albert, *J. Chromatogr. A* **1995**, *703*, 123.
[38] S.X. Peng, B. Borah, R.L.M. Dobson, Y.D. Liu, S. Pikul, *J. Pharm. Biomed. Anal.* **1999**, *20*, 75.
[39] G. Hegy, E. Görlach, R. Richmond, F. Bitsch, *Rapid Commun. Mass Spectrom.* **1996**, *10*, 1894.
[40] D.B. Kassel, M.D. Green, R. Wehbie, R. Swanstrom, *J. Berman, Anal. Biochem.* **1995**, *228*, 259.
[41] R. Richmond, E. Görlach, J.-M. Seifert, *J. Chromatogr. A* **1999**, *835*, 29.
[42] B.D. Duléry, J. Verne-Mismer, E. Wolf, C. Kogel, L. van Hijfte, *J. Chromatogr. B* **1999**, *725*, 39.
[43] E.W. Taylor, M.G. Qian, G.D. Dollinger, *Anal. Chem.* **1998**, *70*, 3339.
[44] J.L.E. Ruebsaet, J.H. Beijnen, A. Bult, R.J. van Maanen, J.A.D. Marchal, W.J.M. Underberg, *J. Pharm. Biomed. Anal.* **1998**, *17*, 955 and 979.
[45] M. Schnölzer, A. Jones, P.F. Alewood, S.B.H. Kent, *Anal. Biochem.* **1992**, *204*, 335.
[46] J.W. Metzger, C. Kempter, K.-H. Wiesmüller, G. Jung, *Anal. Biochem.* **1994**, *219*, 261.
[47] D.R. Goodlett, P.A. Abuaf, P.A. Savage, K.A. Kowalski, T.K. Mukherjee, J.W. Tolan, N. Corkum, G. Goldstein, J.B. Crowther, *J. Chromatogr. A* **1995**, *707*, 233.
[48] V. Sanz-Nebot, A. Garcés, J. Barbosa, *J. Chromatogr. A* **1999**, *833*, 267.
[49] J. Cai, J.D. Henion, *J. Chromatogr. A* **1995**, *703*, 667.
[50] F.Y.L. Hsieh, J. Cai, J.D. Henion, *J. Chromatogr. A* **1994**, *679*, 206.
[51] M.A. Hoitink, E. Hop, J.H. Beijnen, A. Bult, J.J. Kettenes-van den Bosch, W.J.M. Underberg, *J. Chromatogr. A* **1997**, *776*, 319.
[52] R.M. Holt, M.J. Newman, F.S. Pullen, D.S. Richards, A.G. Swanson, *J. Mass Spectrom.* **1997**, *32*, 64.
[53] E. Clayton, S. Taylor, B. Wright, I.D. Wilson, *Chromatographia* **1998**, *47*, 264.