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Improving the detection of degradants and impurities in pharmaceutical drug products by applying mass spectral and chromatographic searching

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Abstract

Liquid chromatography/mass spectrometry (LC/MS) and NMR are commonly used to identify metabolites, impurities and degradation products in the pharmaceutical industry. To more efficiently deal with the large volumes of data these techniques generate, software programs have been developed by various vendors to assist in the identification of these compounds through the use of spectral and chromatographic search algorithms. The feasibility of using such programs for detecting drug degradants and impurities is assessed. A number of compounds encompassing a wide range of both chemical and pharmaceutical properties were tested using LC/UV/MS and the spectral/chromatographic search algorithm MetaboLynx™ (Micromass UK Ltd.) to determine the feasibility of detecting analytes at low concentrations. In addition, drug product and stressed drug substance samples containing quinapril hydrochloride, the active ingredient in Accupril® tablets, were determined by liquid chromatography with atmospheric pressure ionization–time-of-flight (API LC–TOF) and an API LC–quadrupole (Q) mass spectrometer, and the resulting data was processed using MetaboLynx. The ability of this program to detect and list a variety of analytes known to be present in the samples was evaluated. The combination of LC/UV, LC/MS and spectral/chromatographic searching is a valuable tool for the detection of impurities at low levels.

Keywords: MetaboLynx™; LC/MS; Drug product degradants; Quinapril hydrochloride; Accupril®

1. Introduction

Liquid chromatography, in tandem with atmospheric pressure ionization/mass spectrometry (API LC/MS), is commonly used in the pharmaceutical industry for identification of impurities and degradants in drug substances and drug products. Liquid chromatography/mass spectrometry is a selective and sensitive technique that can give both qualitative and quantitative information. Furthermore, it allows for fast method development, and is widely applicable to most compounds of interest to the pharmaceutical industry [1].

Over the last decade, API LC/MS has largely replaced direct probe electron impact and chemical ionization mass spectrometry for structural elucidation.

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This on-line approach also provides an easy means of volatilizing relatively nonvolatile analytes to obtain molecular weight information (MS) and structural information based on collision-induced-dissociation (MS/MS and MS^n). This information, coupled with various NMR techniques allows determination of many types of molecular structures.

In spite of the enormous power of NMR and LC/MS, identification of metabolites, impurities and degradation products is a difficult and time-consuming process. Because of the data intensive nature of full scan LC/MS, considerable time and effort is required to interrogate the data in order to extract the results needed to identify unknowns. In principle, the speed of identification can be improved by applying spectral and chromatographic search programs, such as MetaboLynx™ [2] and Advanced Chemistry Development [3], to aid in the detection and identification of unknowns, particularly those buried in spectral noise.

In drug product chemical-analysis, the industry standard is to identify impurities and degradants at levels 0.05% of the label claim of the active ingredient. The current method of analysis at the early stages of development uses LC in combination with UV, diode-array detector (DAD), and MS detection methods. A common approach is a gradient method where it is shown that the UV response can be quantitated at 0.05% of the active ingredient concentration. This approach can bias results, because only degradation and impurities with a similar or better absorptivity will be observed. The addition of mass spectrometry to drug product analysis improves the ability to detect different types of impurities [4,5].

MetaboLynx is a spectral/chromatographic search program associated with the MassLynx instrument/data management program, developed by Micromass UK Ltd. (Manchester, UK). It has been designed specifically to detect and identify metabolites for in vivo drug metabolism studies [6] but it has also had limited application to the identification of impurities and degradation products [7,8]. MetaboLynx compares the mass spectral chromatograms of a control versus a metabolized (or stressed) sample, and automates the detection, identification and reporting of metabolites.

In this paper, we determine if MetaboLynx can be applied to drug substance and drug product chemistry to help identify degradants and impurities. In addition, we assess whether MetaboLynx can be used to improve the detection of these degradants and impurities beyond simple UV and MS data reduction. Ten pharmaceutical compounds representing a wide range of chemical properties (i.e. mass, ionizability, overall structure) were tested to determine the power of LC/UV/MS/MetaboLynx to detect and identify analytes at low concentrations. These samples were combined and run using a long, simple LC gradient to determine if a generic method could be applied to detect low levels of potential impurities of a variety of pharmaceuticals. We also tested control and stressed samples of a marketed product, Accupril® tablets, and its active ingredient quinapril, using API LC–time-of-flight (TOF) and API LC–quadrupole (Q). Both pure compound and formulated drug product samples were known to contain several degradants [9,10]. The resulting sets of spectra were then searched by MetaboLynx to determine if this software program could identify these drug degradants. Processing capability and ease of data manipulation were explored.

2. Materials and methods

2.1. Reagents and chemicals

ACS grade acetic acid and HPLC-grade acetonitrile and water were purchased from Mallinckrodt (Paris, KY, USA). Reagent grade ammonium acetate was obtained from J.T. Baker (Phillipsburg, NJ), while trifluoroacetic acid (TFA) was purchased from B & J Brand (Muskegon, MI, USA). Reserpine, and the test compounds ibuprofen, (*S*)-6-methoxy-α-methyl-2-naphthalene acetic acid, promethazine, clenbuterol, omeprazole, and bupivacaine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Gabapentin, Accupril tablets (20 mg), and reference standards of quinapril hydrochloride and known degradants present, including quinaprilat and the cyclic impurity, were also tested and were obtained from Pfizer Global Research & Development (PGRD), Ann Arbor Laboratories. The final test sample consisted of photooxidized quinapril (22 g dissolved in 100 ml methylene chloride and irradiated under a Photochemical Reactor for Enhanced Detection (PHRED®, Aura Industries, Staten Island, NY) lamp source at 254 nm for 90 min and was also

obtained from PGRD, Ann Arbor Laboratories). Although the majority of compounds studied here were unrelated to the parent compound tested (quinapril), they were added as artificial impurities to better evaluate the techniques being tested in this work.

2.2. Equipment

The mixture of 10 pharmaceuticals was run on a YMC-Pack ODS AQ column from YMC Inc. (S-3 μm, 120 Å. 150 mm × 4.6 mm; c/o Waters Corp., Milford, MA, USA). In both the photoxidized quinapril and Accupril tablet experiments, the LC separations were performed using a Cyano HSB column from BHK Laboratories (5 μm, 120 Å, 2.0 mm × 100 mm; Naperville, IL, USA).

The electrospray ionization/time-of-flight (ESI/TOF) and both of the electrospray ionization/quadrupole (ESI/Q) mass spectrometry experiments were performed on Micromass (Beverly, MA, USA) instruments. The LCT (TOF), Quattro Ultima (Q), and ZQ (Q) were all equipped with Z-spray electrospray ionization sources. The ZQ quadrupole was used for the mixture of widely ionizable compounds and was attached to a Waters 2690 Alliance HPLC (Milford, MA, USA). A Thermoseparation Products HPLC (Thermo Finnigan, San Jose, CA, USA) was coupled to the TOF, while an Agilent 1100 series HPLC (Agilent Technologies, Wilmington, DE, USA) was connected to the Quattro Ultima quadrupole instrument. The latter two instruments were used for the Accupril and photooxidized quinapril samples.

The mass spectrometers were controlled and data was acquired using the Windows-based software MassLynx, version 3.5 (Micromass). The software program MetaboLynx, version 3.5 (Micromass), was subsequently used to process the data to search for both expected (e.g. quinaprilat and the cyclic impurity) and unexpected degradants.

2.3. Preparation of stock solutions and samples

The first sample consisting of a mixture of 10 pharmaceuticals was prepared by weighing approximately 12.5 mg of ibuprofen, (*S*)-6-methoxy-α-methyl-2-naphthalene acetic acid, promethazine, clenbuterol, omeprazole, bupivacaine, quinapril's cyclic impurity, quinaprilat, and gabapentin each into a 250 ml volumetric flask. A mixture of 50% acetonitrile, 50% water was added to approximately 90% of volume, followed by sonication for 30 min. The solution was allowed to cool to room temperature, and then filled to volume. A stock solution of quinapril was prepared in a similar manner at a concentration of 1 mg/ml. The stock was then diluted to a concentration of 100 μg/ml to serve as a control for the MetaboLynx studies. The mixture stock solution was diluted to 5.0, 2.5, 1.0, 0.5, 0.1, 0.05, and 0.01% of the 100% (100 μg/ml) level of quinapril. The quinapril stock solution was spiked into each of these dilutions to yield the 100% level.

The control for the Accupril tablets and photooxidized quinapril samples consisted of standards of quinapril, quinaprilat, and the cyclic impurity and were separately prepared by dissolving approximately 3 mg in 20 ml of 50% acetonitrile, 50% of 50 mM aqueous ammonium acetate (pH not adjusted) to yield a concentration of approximately 0.16 mg/ml. Because quinaprilat and the cyclic impurity are always present as impurities in the stock solution of quinapril, the three standard solutions were then combined and diluted in 50:50 (v/v) acetonitrile:water to obtain approximately 25 μg/ml of each component.

Ten Accupril tablets (20 mg) were added to a 500 ml volumetric flask and dissolved in 35:65 (v/v) acetonitrile:water. The resulting solution was centrifuged and filtered with a syringe-tip filter (Gelman, Acrodisc CR, 25 mm, 0.45 μm PTFE membrane; Gelman Sciences, Ann Arbor, MI) into a HPLC vial.

The third sample consisted of photooxidized quinapril. Two milligrams of this material was dissolved in 15 ml of 50:50 (v/v) acetonitrile:aqueous ammonium acetate to give approximately 130 μg/ml. All solutions were stored at 5 °C.

2.4. Chromatographic and mass spectrometry conditions

Ten microliters of the spiked mixture samples were injected onto the LC column using gradient conditions from 10% acetonitrile, 90% of 0.05% acetic acid in water from 0.00 to 2.00 min, to 90% acetonitrile, 10% of 0.05% acetic acid in water from 2.00 to 55.00 min. The column was then equilibrated back to the original conditions for 5 min. A flow rate of 1.0 ml/min was used, with an approximate 1:10 split incorporated for the MS. These spiked sample dilutions were injected

onto the LC/DAD/ZQ and analyzed by UV at 214 and 254 nm and by MS in both positive and negative electrospray modes.

Typical source conditions for the ZQ in the positive electrospray mode were as follows: capillary 3.00 kV, cone 60 V, source block temperature 120 °C and desolvation temperature at 400 °C. For ESI in the negative mode, the capillary 3.48 kV, cone 30 V, and the temperatures remained the same as that mentioned in the positive ion mode. Samples were scanned from mass-to-charge ratios (*m*/*z*) 50 to 500.

The Accupril tablets and photoxidized quinapril samples were injected (3 μl) onto the LC column under isocratic conditions using 70% ACN, 30% water, 0.01% of 300 mg/ml aqueous ammonium acetate and 0.006% TFA as the mobile phase. A flow rate of 0.25 ml/min was used for the quadrupole experiments, while 0.70 ml/min with a 1:1 split was employed for the TOF experiments.

Typical ion-source conditions for the TOF experiments in the ESI positive mode (ESI+) were as follows: capillary voltage 3.5 kV, sample cone 55.0 V, extraction cone 10.0 V, RF lens 300 V, desolvation 400 °C and source temperature 100 °C. For experiments in the negative ionization mode (ESI−) the sample cone was at 60 V, the RF lens at 200 V, and the extraction cone at 2 V, while the temperatures were the same as for the positive ion mode. The diode-array detector was monitored at 214 nm for the TOF experiments. In addition, reserpine (500 pg/μl in 1:1 acetonitrile:water + 0.2% formic acid) was used as a lock-mass compound and infused using a syringe pump to allow for exact mass determinations.

Typical source conditions for the quadrupole in the positive electrospray mode were as follows: capillary 4.0 kV, cone 70 V, source block temperature 150 °C, and desolvation temperature at 400 °C. For ESI in the negative mode, the capillary was at 3.0 kV, cone voltage at 70 V, and the temperatures remained the same as that mentioned in the positive ion mode. Samples were scanned from *m*/*z* 100 to 500 on both instruments.

3. Results and discussion

3.1. MetaboLynx method parameter optimization

The value of MetaboLynx in finding unknowns buried in spectral noise as well as enabling fast and easy data handling was explored. A chromatographic data set consisting of full scan spectral data can contain upward of several million data points, depending on the length of the chromatographic run and the spectral density (number of scans/min, number of data points per scan). Data sets collected by LC/TOF will generally be much larger than those collected from a scanned quadrupole because the scan speed of the TOF is much faster. In either case, the sheer size of these data sets makes the task of manually finding those peaks with the correct mass-to-charge ratios (*m*/*z*) difficult, especially if there are several *m*/*z* of interest in numerous samples. The use of computerized programs offers some help in processing and interrogating these data sets by automatically looking for peaks with known *m*/*z* and by summarizing and reporting these results [8].

In MetaboLynx, data is processed through the sample list page of MassLynx. Here, the data file is identified as either a control (usually a standard of the parent compound, here quinapril) or an analyte (sample containing metabolites or degradants, here the serial dilutions of the mixture of 10 compounds, Accupril tablets, or the photoxidized sample). The exact mass or the molecular formula of the parent is also required for input into the sample list. Finally, the user creates a method file, whereby, a variety of parameters are specified and customized, and then selects it in the sample list page. These parameters (e.g. chromatographic integration and spectral thresholds, tolerances, step size) are used to identify differences between controls and samples with respect to the intensities, *m*/*z* and retention time.

In the method file, both positive (e.g. M + H) and negative adducts can be chosen to aid in identification. In addition, the analyst can specify a list of potential or expected impurities. The user can create a novel list or import an external database. For example, the result list from an expert system, such as Computer Assisted Mechanistic Evaluation of Organic reactions (CAMEO [11]), can be utilized. Alternatively, the existing library within MetaboLynx can be employed. Because MetaboLynx was developed for searching for biotransformation products, the resident library consists of components from common metabolic pathways, such as oxidation, deamidation, glycosolation, and methylation. It is also possible to program MetaboLynx to search for unknown/unexpected

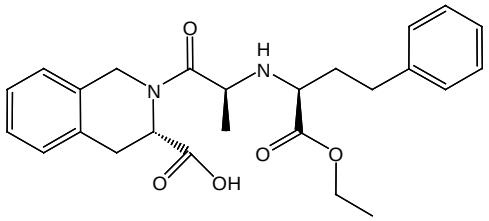


Fig. 1. Chemical structure of quinapril (3-isoquinolinecarboxylic acid, 2-[2-[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-, monohydrochloride, $C_{25}H_{30}N_2O_5ClH$).

components (i.e. those created by nonmetabolic processes or compounds not included in an imported library) that are found by mass spectral comparison of controlled versus reacted/metabolized samples. In order to do this, the user must specify the scanning range (e.g. 100–500 amu) and the size of the step scan (e.g. 1–10 amu).

Parameter selection in the method file is key to finding both expected/known/listed and unexpected/unknown/unlisted components. We define a good result as one in which all of the degradant peaks are integrated, and the degradants are listed in the report as found. Furthermore, a minimum number of peaks that can be attributed to background noise are integrated and listed in the report. Correct parameter selection was found to be more complex than anticipated and was a trade-off between the different parameters. The authors found that parameters needed to be adjusted for each sample, and we were unable to create a generic method for MetaboLynx.

The Browser function is used to view the results generated by the MetaboLynx method file. Any peaks in the sample that do not match with peaks in the control, according to the criteria specified in the method, are regarded as being metabolites/degradants and are added to the output last for found components.

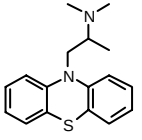
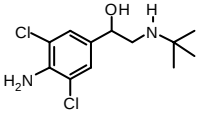
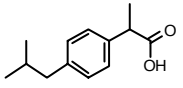
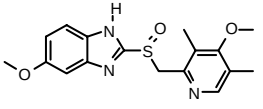
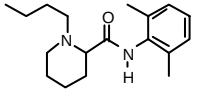
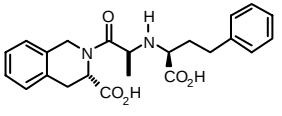
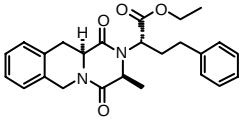
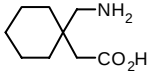
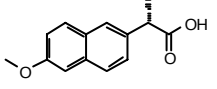
3.2. Low-level spiking studies

The first sample assessed consisted of a mixture of nine analytes serially diluted from 5.0 to 0.01 μg/ml. These levels represented 5.0–0.01% of the quinapril (Fig. 1) 100% level concentration (100 μg/ml), which was spiked into each sample dilution to yield 100 μg/ml. The structures of these compounds are shown in Table 1. Using the 10–90% acetonitrile 60-min gradient and both positive and negative ion electrospray to test the potential of Metabolynx to find analytes, two runs were made. For the first run, all analytes were included as “expected” within the MetaboLynx method. For the second run, no analytes were listed as “expected” and MetaboLynx was tested to see if all analytes were found and reported. We then compared the success of MetaboLynx in finding and reporting the analytes of interest with the peaks that could be visually observed in the UV data, the total ion chromatogram (TIC) and the respective extracted ion chromatograms (XIC) generated from the full scan in MassLynx.

Even though many of the compounds were, potentially, capable of ionizing in the negative ion mode, peaks at these low levels were not observed. Therefore, all of the results discussed are those from the positive ion mode. MetaboLynx performed very well in finding and reporting the masses of the components of interest when they were listed as expected in the method. Table 1 summarizes results of four determination techniques: UV at the commonly used wavelengths of 210 and 254 nm, TIC from the full ESI(+)/Q scan, the XIC generated in MassLynx from the full scan, and the compounds listed as found in the MetaboLynx report.

MetaboLynx reported peaks for quinaprilat, promethazine, bupivacaine, and gabapentin at a lower dilution level than that which was detected or could be visually observed (no distinguishable peak in an expanded chromatogram) in the UV data, the compounds’ respective XIC, and the electrospray positive TIC. Gabapentin has no chromophore, elutes very close to the void and, thus, was not detected by UV. It was only visually observed at 5.0 and 2.5% levels in the TIC, but due to the peak’s position, an analyst could easily overlook the peak. In the XIC, the peak was observed at the 1.0% level, whereas MetaboLynx was able to extract the mass of interest out of the noise and report it as found at the 0.5% level. Promethazine results were improved by a factor of five with MetaboLynx relative to UV and TIC. Quinaprilat and bupivacaine results were improved 10-fold with the use of MetaboLynx (0.05%) relative to those from the UV (0.5%; 50 times lower for bupivacaine at 254 nm), and by 20 times compared to the TIC. All three compounds were observed at the same level in the user generated XIC, but in all cases the peak was much smaller (S/N 5.3 versus 26.5,

Table 1
Summary of UV, total ion chromatogram (TIC), extracted ion chromatograms (XIC), and MetaboLynx results of the sample mixture of widely ionizable components, with quinapril spiked into the dilution levels at the 100%, or 100 μg/ml, level

Name	Structure	MWT	Retention time (min)	Limit of detection			
				UV	TIC	XIC	MetaboLynx
Promethazine		284.14	16.50	2.5% (254 nm), S/N = 15.4; 2.5% (210 nm), S/N = 3.3	2.5%, S/N = 3.5	0.5%, S/N = 5.3	0.5% (reported), S/N = 26.5; 0.1% (visual), S/N = 2.8
Clenbuterol		276.08	8.77	0.5% (254 nm), S/N = 25.3; 1.0% (210 nm), S/N = 2.0	2.5%, S/N = 3.0	0.5%, S/N = 3.4	1.0%, S/N = 5.8
Ibuprofen		206.13	2.40	ND	ND	ND	ND
Omeprazole		345.11	14.97	0.1% (254 nm), S/N = 24.7; 0.5% (210 nm), S/N = 6.8	ND	ND	ND
Bupivacaine		288.22	12.60	2.5% (254 nm), S/N = 3.2; 0.5% (210 nm), S/N = 2.9	1.0%, S/N = 4.1	0.05%, S/N = 2.4	0.05%, S/N = 11.2
Quinaprilat		409.18	17.30	0.5% (254 nm), S/N 12.2; 0.5% (210 nm), S/N = 3.1	1.0%, S/N = 2.2	0.05%, S/N= 1.6	0.05%, S/N = 5
Quinapril's cyclic impurity		420.20	33.05	<0.05%	<0.05%	<0.05%	<0.05%
Gabapentin		171.13	2.10	ND	2.5%, S/N = 3.3	1.0%, S/N = 2.7	0.5%, S/N = 1.1
(*S*)-6-Methoxy-α-2-naphthalene acetic acid		230.09	27.45	0.1% (254 nm), S/N = 8.96; 0.5% (210 nm), S/N = 9.9	2.5%, S/N = 1.2	2.5%, S/N = 3.8	1.0%, S/N = 3.0

The results listed below give the lowest spiked level that could be visually differentiated from that of the background noise, followed by an approximate signal-to-noise (S/N)) value. The MetaboLynx results were reported, unless otherwise specified (ND: not determined).

1.6 versus 5.0, and 2.4 versus 11.2, respectively) and the chromatogram was much noisier than that in the MetaboLynx generated XIC.

Fig. 2 shows the results for bupivacaine, which has a retention time of 12.6 min, at the 0.05% level. The MetaboLynx-generated chromatogram (Fig. 2D) contains less noise compared to the extracted ion chromatogram from MassLynx (Fig. 2B), significantly increasing the signal-to-noise of the bupivacaine peak from approximately 2.5 to 11. In addition, MetaboLynx reported the component found and listed it in the summary table. The peak cannot be observed visually in either the UV chromatogram or the MS full scan, and would likely be overlooked by an analyst using the industry standard methods. In this case, MetaboLynx would have been a beneficial tool to employ in conjunction with the UV and MS. Otherwise, this component would not have been found if the analyst relied solely on the UV or full scan MS data.

The UV data gave better results than the other methods for three compounds: (*S*)-6-methoxy-α-2-naphthalene acetic acid, clenbuterol, and omeprazole. The

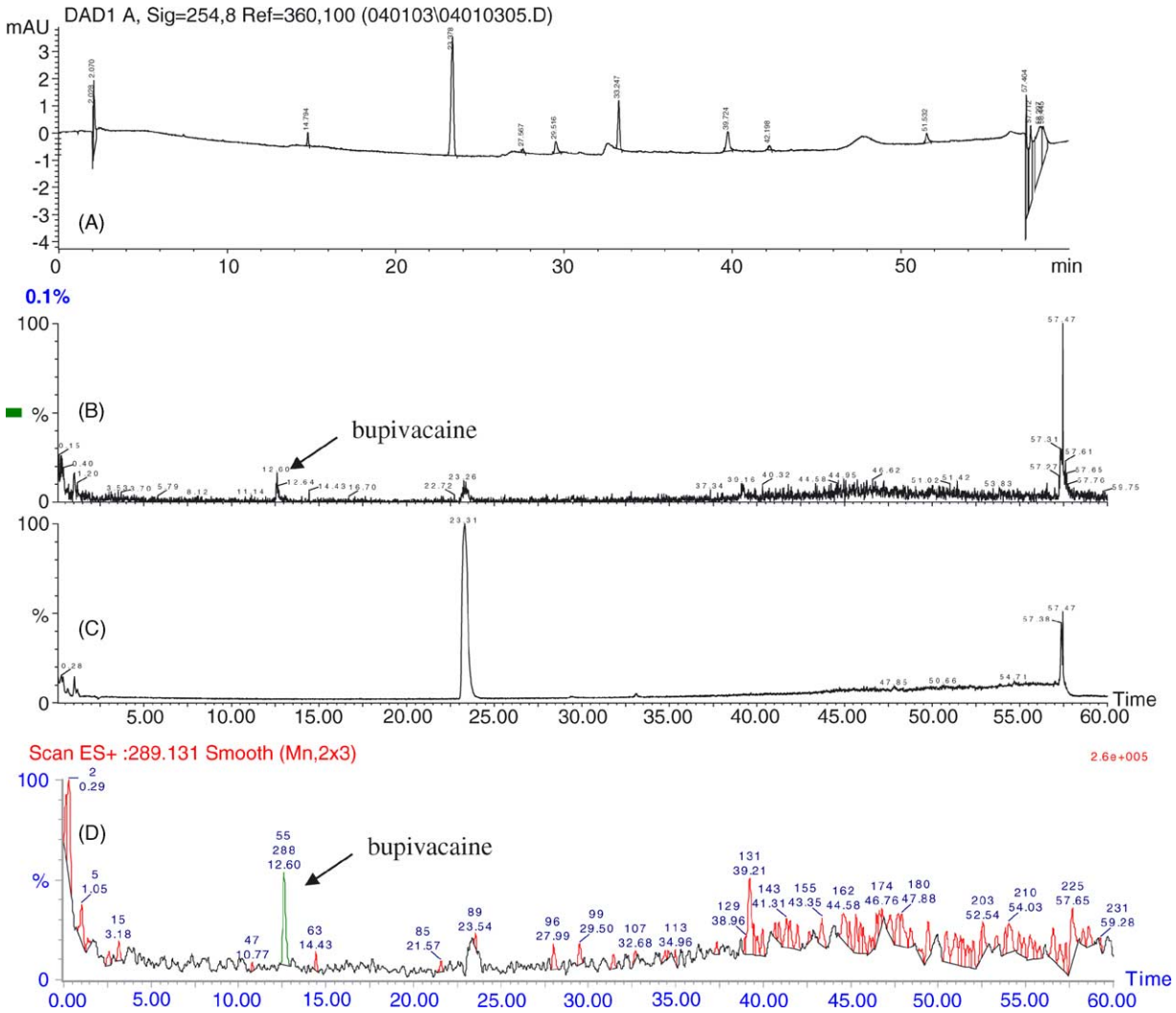


Fig. 2. Data generated from the 0.1% level dilution of the sample mixture of nine pharmaceutical components spiked with quinapril at the 100% level: (A) the UV trace at 254 nm; (B) extracted ion chromatogram corresponding to the M + 1 value of bupivacaine extracted from the full ESI(+)/Q scan; (C) full ESI(+)/Q scan; (D) MetaboLynx-generated extracted ion chromatogram for bupivicaine.

naphthalene acetic acid analogue was observed at a lower level in UV, followed by MetaboLynx (listed component), which was in turn two and a half times lower than both the XIC and TIC results. However, once again, MetaboLynx enhanced detection over that of visual observation of the MS data. Ibuprofen was not determined at the spiking levels used in this study by either UV or MS.

When the analytes were not specifically listed in the method file as expected, MetaboLynx did not perform as well in finding and reporting them. MetaboLynx did extract the analytes and generated chromatograms showing that they were present. However, they were not integrated or listed in the MetaboLynx final report. In order for an analyst to find the "unknown" analytes it was necessary to scroll through the total MetaboLynx output (450 chromatograms). Despite numerous attempts at altering the various integration and threshold values, numerous peaks that appeared to be background noise were integrated, whereas those of interest were not, but the peaks were still observed. Even though this appears similar to just extracting 1 amu chromatograms from a TIC using MassLynx, as previously mentioned, the MetaboLynx-generated XIC and step scans had at least a five times improvement of signal-to-noise over those generated by MassLynx. Therefore, analyte peaks that were previously buried in the noise were only observed by using MetaboLynx.

In this study, MetaboLynx was shown to benefit the detection of low-level analytes. It gave improved results over using UV alone for four of the analytes tested here, whereas only three analytes gave improved results when two commonly used detection wavelengths were assessed from the diode-array data. The resulting step scan chromatograms generated by MetaboLynx contained less background noise than those from an analyst generated extracted ion chromatogram in MassLynx. Therefore, even if MetaboLynx did not list all of the peaks of interest in its report, the analyst could scroll through the step scans to look for peaks of interest, and have a better chance of finding them than manual extraction from the full scan data.

3.3. MetaboLynx success at finding degradants

Next, we applied MetaboLynx to two samples whose degradant profiles had previously been assessed using LC/UV. The assayed Accupril tablets were known to contain the well-characterized degradants quinaprilat and the cyclic impurity [9]. In the photooxidized lot of quinapril, six degradants were known to be present at 0.08–1.19% of the parent drug [10].

Using MetaboLynx, we were able to locate both degradants in the assay tablets in the positive electrospray/quadrupole data files. As expected, only the quinaprilat was found in negative ion mode, because the cyclic impurity could not be negatively ionized.

Fig. 3 shows the results from the photoxidized degradation sample. As observed above, despite method optimization, the degradants were not always integrated and/or reported by MetaboLynx (Fig. 3E). This was especially true when the component in question eluted at or near the solvent front and large step sizes (5–10 amu) were used. In order to integrate all the known degradation products it was necessary to reduce the step size to 1 amu. Even with this small step size, peaks corresponding to all degradation products were integrated but not reported, forcing a visual inspection of 450 chromatograms.

Fig. 4 are the extracted chromatograms of the marketed drug product, and is a specific example of why careful parameter selection is vital for both expected/listed and unexpected/unlisted components. Here, a sample extract from Accupril tablets was injected into the ESI(+)/Q system and subsequently processed by MetaboLynx using a method that that been previously optimized for this sample. First, a degradant with a mass of 410.18 was listed in the expected components section of the method file. MetaboLynx found a peak with this mass, generated an extracted ion chromatogram (Fig. 4A) and listed it in the results table as a found component. Next, this same data file was processed under identical conditions, with the exception that this mass was not listed in the expected components table of the MetaboLynx method. Only one peak at 1.11 min was detected (corresponding to the cyclic impurity), however, the quinaprilat peak was clearly observed but not integrated or reported (Fig. 4B). Although significantly lowering the signal-to-noise threshold in the metabolynx program resulted in the detection of this peak, it also resulted in hundreds of additional background noise peaks also being reported. To minimize this effect visual inspection may be required for accurate assessment of unexpected/unlisted components.

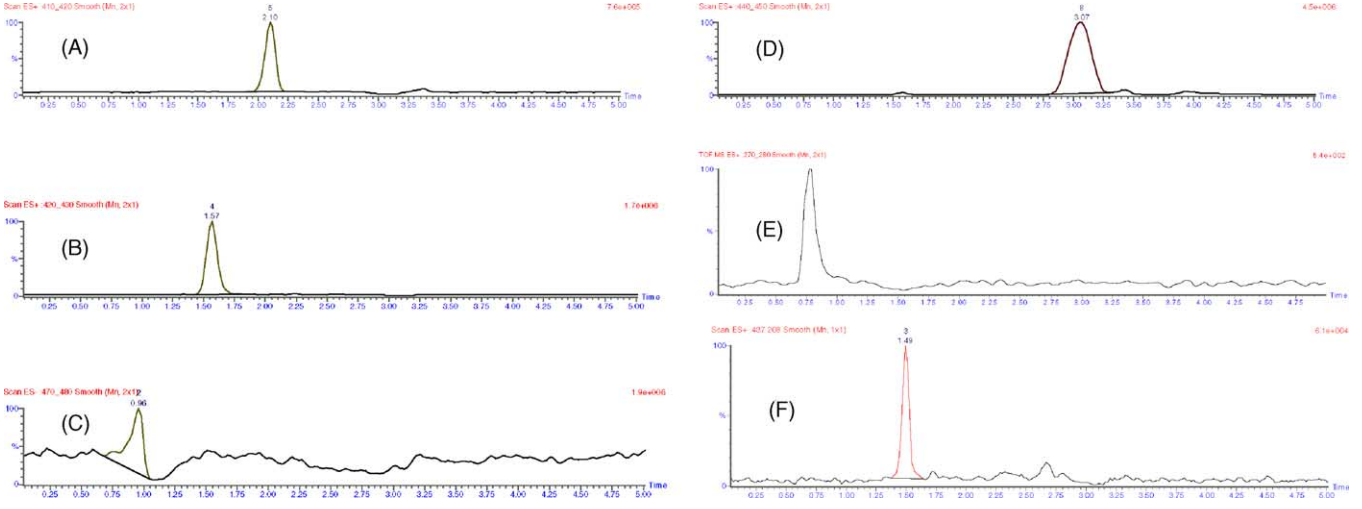


Fig. 3. Step scans (unexpected/unlisted components) from MetaboLynx depicting the presence of a peak corresponding to degradants: (A) ESI(+)/Q of quinaprilat; (B) ESI(+)/Q of the cyclic impurity; (C) ESI(−)/Q of Degradant 4; (D) ESI(+)/Q of Degradant 5; (E) ESI(+)/TOF of Degradant 6: (F) the resulting extracted ion chromatogram generated by MetaboLynx for Degradant 3, as determined by ESI(+)/Q and listed in the method file as an expected metabolite.

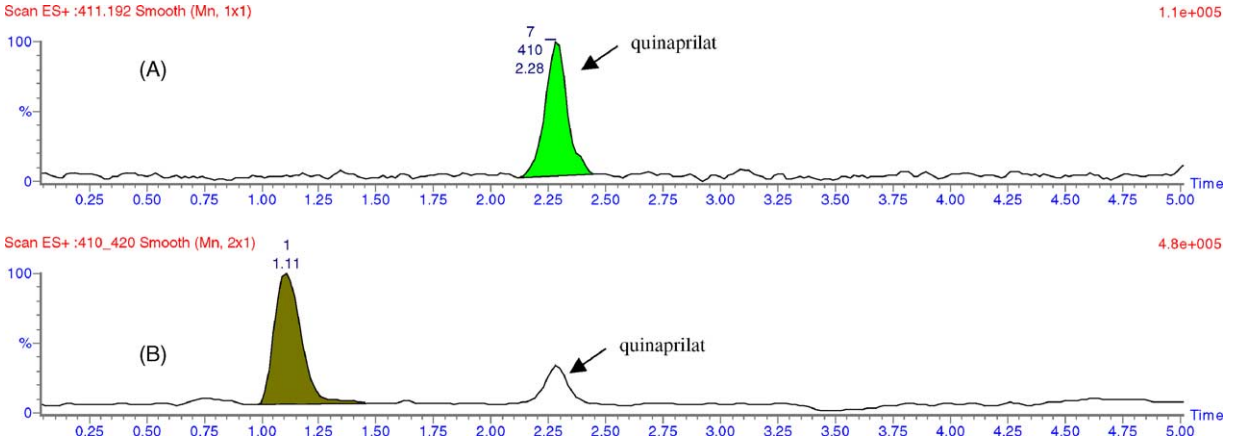


Fig. 4. ESI(+)/Q determinations of a sample of dissolved Accupril tablets: (A) the resulting extracted ion chromatogram generated by MetaboLynx when the known degradant was included in the "expected" component table; (B) the resulting step scan generated by MetaboLynx when the known degradant was an "unexpected" component, the peak at 1.11 corresponds to the cyclic impurity, (MW = 420.2); and the quinaprilat is not integrated.

The photooxized sample also showed that MetaboLynx could detect unknown analytes buried in background noise. When the photooxidized sample was evaluated by ESI(−)/Q, a peak corresponding to Degradant 4 (expected retention time of 1.7 min) was not observed in the resulting total ion chromatogram (Fig. 5A). Furthermore, MetaboLynx did not integrate any peaks in the corresponding 10 amu step scan chromatogram. However, when the step size was reduced to 1 amu, a peak was more easily observed and was integrated by the software program (Fig. 5B). Without the assistance of MetaboLynx, this small, but significant peak could have been easily overlooked. It was not reported, however, and it would be necessary to scroll through the 450 generated step scans to observe this impurity.

3.4. MetaboLynx applied to ESI/Q versus ESI/TOF

The ability to easily determine the exact mass (± 0.0005 amu) of a compound from LC/MS is a powerful tool when trying to identify or confirm the

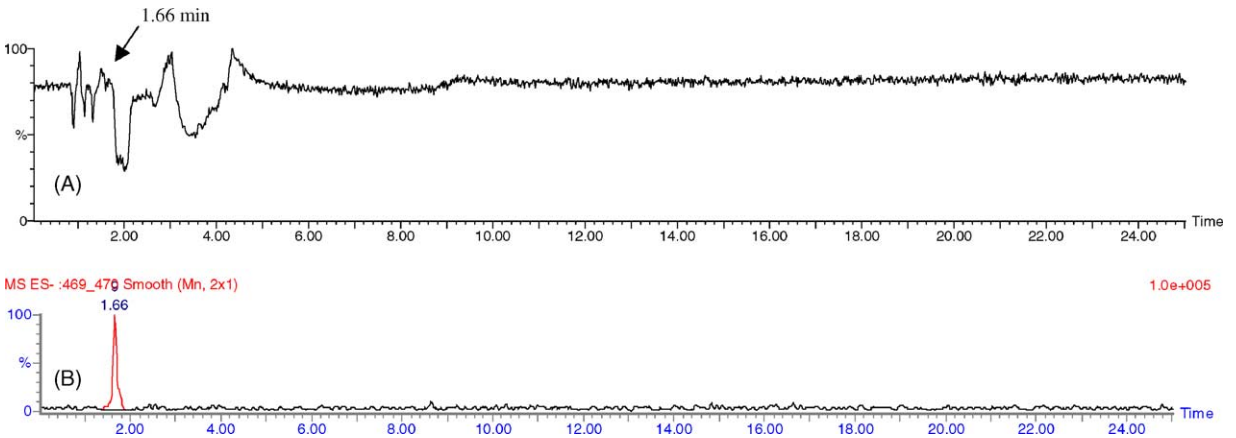


Fig. 5. ESI(−)/TOF determination of dissolved Accupril tablets: (A) the TIC (*m*/*z* 150–500); (B) the resulting step scan generated by MetaboLynx showing the peak of interest.

presence of a degradant. If a component is expected, a spectrum that gives the mass of a compound to within 5 ppm of the calculated molecular mass gives a high level of confidence to its presence, and its elemental composition. Nominal mass measurement (± 0.5 amu) on a quadrupole does not give this information. The MetaboLynx method file allows accurate mass spectra to be used. Therefore, in order to compare the applicability and ease of use of the applications manager with both the TOF and quadrupole, the quinapril hydrochloride samples were analyzed with both types of instruments and the resulting data processed by the software program.

In these studies, MetaboLynx was successfully applied to both types of MS. The MetaboLynx method was found not to be directly transferable from the quadrupole to the TOF, possibly because the character of signal to noise was widely different. It was necessary to adjust most of the MetaboLynx method parameters when processing data generated by the two types of instruments. In addition, the data files generated by the TOF in these studies were approximately 15–20 times larger than those generated by a full scan of the quadrupole. Therefore, processing of the TOF data required several times more computer network resources and it was necessary to transfer the TOF data from the network server to the local hard drive in order to process. Fewer "hits" were obtained with the TOF experiment compared to scanned quadrupole data.

4. Conclusion

MetaboLynx can be used in drug substance and drug product analysis to detect degradants and impurities that are not observed visually by LC/UV/MS. MetaboLynx enhanced signal to noise ratios for most compounds relative to either LC/MS or LC/UV. It was shown that impurities present in Accupril and stressed quinapril samples were successfully detected by MetaboLynx using data from either API LC–TOF or API LC–quadrupole instruments.

Some drawbacks of MetaboLynx were that the method was time-consuming to optimize, processing the larger data files with small step size did result in computer system failures, and not all detected and integrated degradants were listed in the final report. In some cases it was necessary to visually inspect numerous step scans to check for peaks of interest. However, the program helped detect analytes at concentration levels necessary for drug product analysis. In addition, MetaboLynx reduces the noise, increasing the likelihood of the peak of interest being observed relative to an extracted ion chromatogram generated in MassLynx alone.

The major benefit of MetaboLynx is the ability to identify unknown degradants buried in the noise of a sample mass chromatogram. The application of this program to a compromised sample (forced degradation, stability, accelerated stability, etc.) would help detect impurities not observed by an analyst in either the MS full scan or the UV chromatogram. In drug product characterization, degradation products $\geq$0.1% need to be detected and tracked. At present, LC/UV methods are used for this purpose. Impurities without chromophores would not be detected with LC/UV at this concentration level. Furthermore, an impurity with a very low extinction coefficient that elutes near the void or underneath a much larger peak could easily go unnoticed in a DAD, even using peak purity software. The combination of LC/UV, LC/MS, and MetaboLynx is useful for detecting impurities.

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References

[1] D.T. Rossi, M.W. Sinz, Mass Spectrometry in Drug Discovery, Marcel Dekker Inc., New York, 2002 (Chapter 2).
[2] Micromass UK Ltd.; www.micromass.co.uk under Home then Software, MetaboLynx.
[3] http://www.acdlabs.com/products/spec_lab.
[4] E.L. Simmonds, W.J. Lough, M.R. Gray, J. Pharm. Biomed. Anal. 32 (2003) 1113–1122.

[5] D.W. Reynolds, et al., Pharm. Tech. (February) (2002) 48.
[6] J. Castro-Perez, J. Kirby, S. Preece, Am. Biotechnol. Lab. 18 (2000) 38, 42, 44.
[7] S. Monte, J. Castro-Perez, S. Preece, Automated processing of LC/MS data for the detection of drug impurities and degradants in the pharmaceutical industry, Presented at the 17th Montreux Symposium, Montreux, Switzerland, November, 2000.
[8] S. Monte, J. Castro-Perez, S. Preece, Application Note 247, Micromass UK Ltd., Manchester, UK.
[9] A.R. Kugler, S.C. Olson, D.E. Smith, J. Chromatogr. B. 666 (1995) 360–367.
[10] Pfizer Global Research & Development Internal Documents.
[11] W.L. Jorgensen, E.R. Laird, A.J. Gushurst, J.M. Fleischer, S.A. Gothe, H.E. Helson, G.D. Paderes, S. Sinclair, Pure Appl. Chem. 62 (1990) 1921–1932.