Drug Discovery and Development

Accelerated Impurity Profiling Workflow for Bulk Drugs and Formulations using an Accurate Mass Workhorse

Suma Ramagiri, Loren Olson, Gary Impey, Joe Fox
AB SCIEX, Concord, Ontario and Framingham, Massachusetts

Key Challenges of Impurity Profiling Workflow

- Identification and quantification of trace-level impurities in drug substances and drug products
- Laborious workflows for accurate characterization and confirmation of related impurities and other degradants which are present at or below 0.1%
- Manual and time consuming structure elucidation process for drug related impurities or unknown impurities and MS/MS fragment assignment

Key Benefits of Impurity Profiling Workflow using TripleTOF™ 4600 System and ImpurityPilot Software

- Simultaneous identification, characterization, batch to batch comparison including trace level impurities using high resolution accurate mass spectrometry (HRMS) and ultra-performance liquid chromatography (UPLC) conditions all in single injection
- Triple quadrupole level sensitivity and quantitative performance for impurities that at or below 0.1%
- Real time mass defect filter acquisition method for identification and confirmation of trace level impurities that are related to active pharmaceutical ingredients
- Easy to use structure elucidation software tool for MS/MS fragmentation interpretation and unknown compound identification
- Seamless characterization and comparison across multiple batches using all in one integrated single software solution

Key Features of ImpurityPilot™ Software and TripleTOF™ 4600 System

- Low mas (<200 Da) resolution at 25,000 -20,000 for accurate fragmentation interpretation at UPLC speed
- Accurate Isotope pattern for elemental composition assignment
- Automated and intelligent peak finding algorithms such as isotope pattern, PI, NL, dynamic background subtraction for both related and unknown impurities with less false positives
- Automated formula assignment and structure prediction using intuitive UI
- In-built ChemSpider search tool for identification of unknown impurities and also access to local compound database
- Correlate and compare impurity trends across multiple batches
- Response factor correction between Diode array detector (DAD/UV) data and MS data
- Report results for each individual and total impurity levels in % peak areas

Introduction

Identification, characterization and quantification of impurities have become an important part of product development process in pharmaceutical industry. An impurity in a bulk drug or formulation as defined by the International Conference on Harmonization (ICH) Guidelines is any component of the drug substance that is not the chemical entity defined as the drug substance/bulk drug and affects the purity of active ingredient or drug substances. Similarly, an impurity in a drug product/formulation is any component of the drug product that is not the chemical entity defined as the drug substance or an excipient in the drug product. These impurities are formed during manufacturing process or formulation process, or upon aging. They include organic compounds that are process related or product related such as starting materials, intermediates, by products, reagents and catalysts. Upon aging several degradation products are formed by ester hydrolysis, oxidative degradation, photolytic cleavage or decarboxylation. The presence of these unwanted impurities even in smaller amounts can affect the efficacy and safety of the pharmaceutical products. According to the ICH guidelines, the new drug substance/product specification should include, where applicable, limits for organic impurities as follows

- Each specified identified impurity
- Each specific unidentified impurity at or above 0.1%
- Any unspecific impurity, with a limit of not more than 0.1%
- Total impurities

In this application note, we describe the use of the AB SCIEX TripleTOF™ 4600 system, an accurate mass workhorse for simultaneous identification, characterization and quantification of simvastatin and its related and unknown impurities as low as 0.1% with UV correction factor and ImpurityPilot™ 1.0 Beta software for data processing

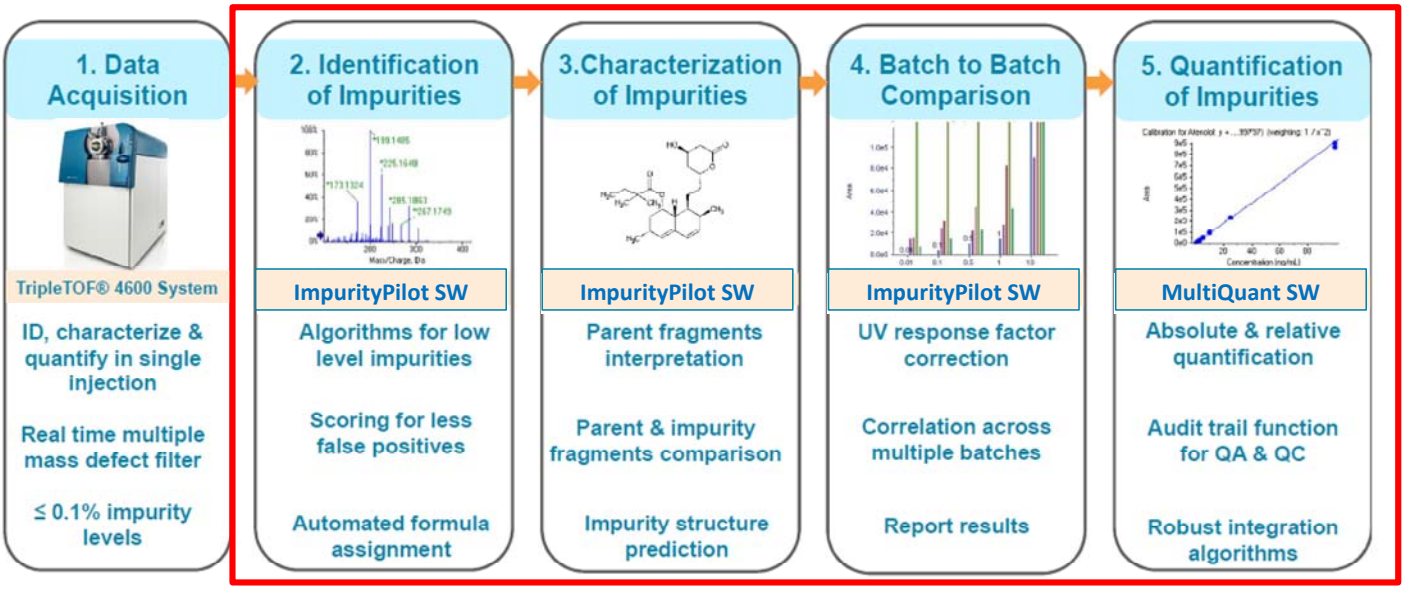


Figure 1: Impurity profiling workflow showing five steps starting from 1. Data acquisition using TripleTOF™ 4600 system 2. Identification of impurities 3. Characterization of impurities 4. Batch to batch comparison and 5. Quantification

Experimental

Sample Preparation:

Five different batches of simvastatin were prepared by spiking its related impurities (table 1) ranging from 0.01% to 10 %. A separate batch was created by spiking equal concentration of simvastatin and its related impurities to evaluate UV response factor and later used for correcting MS signal.

UPLC Conditions:

Sample analysis was performed using Agilent 1290 UPLC system equipped with a diode array detector (DAD). Methanol / water / 0.1% formic acid gradient was used as mobile phase on

a Phenomenex PFP column , 2.1x100 mm, 2.6 µm column. The flow rate was 0.4 mL/min and the column was heated to 40°C.

UV Conditions:

Agilent 1200 DAD detector was set 220-290 nm with PW >0.025 min and acquisition rate > 20 Hz

Mass Spectrometry Conditions:

TripleTOF 4600 System was operated in positive electrospray mode using a DuoSpray™ source. The data acquisition method consisted of a TOF MS survey scan (m/z 100 – 1000) followed by 10 TOF MS/MS dependent scans (m/z 100 – 1000). The TOF MS scan data was used for identification and quantification while the MS/MS data triggered using Information Dependent Acquisition (IDA) was used for confirmation and structure elucidation of related and unknown impurities detected.

Step 1: Data Acquisition

Real Time Multiple Mass Defect Filter (RT-MMDF)

Mass defect filtering (MDF) has been shown to be a powerful tool in identifying compounds that are similar in structure and elemental composition to the parent compound. Traditionally MDF has been performed as a two-step process where full scan data is acquired first, then MDF analysis is performed post processing to identify peaks of interest. A second injection is then performed to acquire the MS/MS spectra of these potential related impurities. To summarize

- RT-MMDF is applied during data acquisition and is separate from data processing algorithms
- RT-MMDF eliminates MS/MS triggering on background noise in addition to real time dynamic background subtraction
- Determines which ion(s) are significantly changing with time and select the best ion(s) to target for MS/MS even if they are low levels
- RT-MMDF is not exclusive but is priority based, so if there is any unrelated impurities like adulterants, contaminants etc. that doesn't fall under specified mass defect range can still be triggered based on other criteria like intensity
- Part of information dependent data acquisition (IDA) logic

Step 2: Identification and Confirmation of Impurities

All five batches of simvastatin were processed using MetabolitePilot™ (MP) Software to identify, confirm and characterize impurities spiked at different concentration levels. All the samples were processed for both MS and UV signals simultaneously in batch mode. Each sample is compared against two different controls, mobile phase control and sample reconstitution solution control to eliminate any background ions as potential impurities.

Intelligent Peak Finding Algorithms for both Related and Unknown Impurities

Multiple algorithms were used simultaneously to identify both related impurities and unknown impurities as low as 0.1%. MP software employs generic peak finding algorithm with dynamic background subtraction to identify unknown impurities. In addition to generic peak finding algorithm, multiple mass defect filtering, isotope pattern matching, and finding metabolites based on common product ions or neutral losses can all be used simultaneously to identify related impurities. Degradation products arising from single bond cleavage like hydroxyl, methyl, ethyl etc. or two bonds cleavage can also be identified using another powerful feature called cleavage metabolites to increase the data processing efficiency. For example in Simvastatin batch 10, MP software identified a total of 11 impurities; of them 5 are related impurities and 6 unknown impurities. Figure 2 is showing the results window with mass accuracy, UV and MS signal with peak area and % peak area for all the impurities.

Compound Name	Molecular Formula	(M+H)+	Mass accuracy (ppm)	Confirmation Score (%)
Simvastatin Parent	C25H38O5	419.2792	0.7	92.1
Lovastatin-Imp1	C24H36O5	405.26355	-0.1	88.2
Dehydro Simvastatin Imp-2	C25H36O4	401.26864	-0.3	92.6
OH Methyl Simvastatin Imp-3	C25H38O6	435.27412	0.4	87
Simvastatin OH Acid Imp-4	C25H40O6	437.28977	-1.5	89.1
Dimer Imp-5	C50H76O10	837.55113	0.6	79

Table 1: Showing list of simvastatin and its related impurities molecular formula and monoisotopic mass, mass accuracy and confirmation score (acceptable confirmation score >60%)

Less False Positives using Confirmation Scoring

When a potential impurity is found in the sample of interest, the software assigns a confirmation score that indicates the likelihood that the peak found is an impurity based on four different criteria like mass accuracy, isotope pattern, mass defect and fragmentation pattern matching with parent molecule. Each impurity is assigned a scored based on above four criteria and can be used to filter the data when reviewing the results. An acceptable confirmation score for impurities would be >60%.

Automated Formula Assignment for Impurities

Each related and unknown impurity is automatically assigned with an elemental composition based on precursor ion mass and fragment ion masses. The ability to assign a formula using combination of precursor and fragment masses gives higher confidence in data quality by reducing number of unrelated chemical formulae for a particular impurity. For example, just based on TOF MS mass accuracy about 10 different chemical formulae were assigned to Lovastatin Imp-1 and number one hit was incorrect, when TOF MS/MS data was added to the formula assignment, the number one hit was the correct formula which is C24H36O4. The ranking of potential chemical formulae is based on mass accuracy, isotope pattern matching and number of matching fragments.

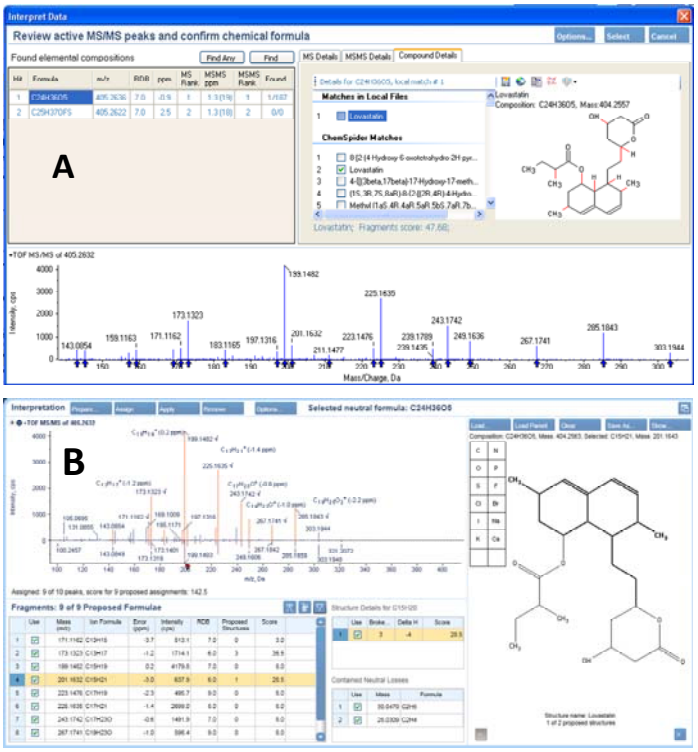


Figure 3: A) Showing elemental composition assignment based on TOF MS, TOF MS/MS and isotope pattern and ChemSpider search results for Lovastatin impurity. Each fragment ion is assigned with elemental composition

Figure 2: Simvastatin batch 10 impurity results table showing list of both related and unknown impurities, correlating MS and UV signal, % peak areas ≤ 0.1.

Step 3: Characterization of both Related Impurities and Unknown Impurities

Both related and unknown impurities can be characterized using MP software structure interpretation module. This integrated structure interpretation functionality allows scientists to quickly evaluate fragmentation pattern of parent, related impurities and propose structures for unknown impurities in an interactive and user friendly manner. When reviewing potential impurities, scientists can edit structures, and perform automatic fragment assignment of proposed structure for any unknown impurity. Both the structure and fragment assignments are saved in the results table and directly accessible in data review.

Figure 4 shows formation of related impurity Lovastatin-Imp1 by demethylation from Simvastatin parent compound. There is a loss of methyl group and fragments interpreted shows similarity with simvastatin parent compound.

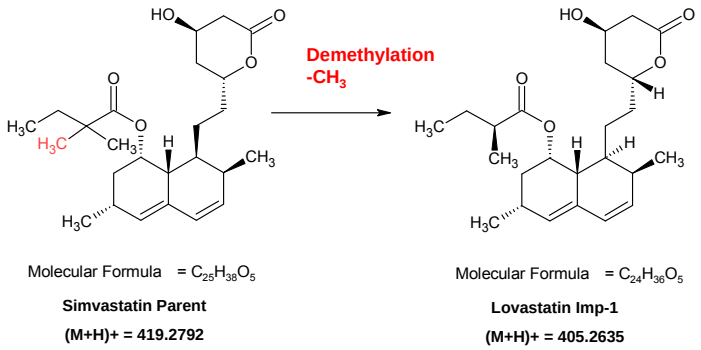


Figure 4: Lovastatin Imp-1 formation from simvastatin

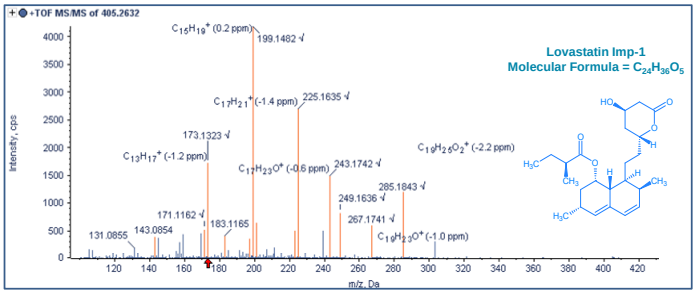


Figure 5: Showing fragmentation interpretation of Lovastatin Imp-1, orange colored peaks are the common product ions from Simvastatin parent compound

Additional Tools for Unknown Impurity Structure Search

Further identification and structure search especially for unknown impurities can be facilitated by software tools like FormulaFinder. Each assigned formula can be searched for a possible chemical structure in both public databases like PubChem, ChemSpider, NIST etc. and also local dataset like in-house chemical library. For example, in simvastatin batch 10, an unknown impurity with m/z was identified at RT 6.0 min with peak area 1.3%, according to ICH guidelines any unknown imp as low as 0.1% should be further investigated to identify and characterize. Using FormulaFinder functionality, elemental composition was assigned as C24H38O4 (m/z 391.2837). Further structure search against ChemSpider, NIST databases resulted in dioctyl phthalate (DOP) as most possible hit. DOP is a plasticizer found in bulk drug storage containers and one of the potential contaminant. There is a higher possibility of DOP contamination in simvastatin batch.

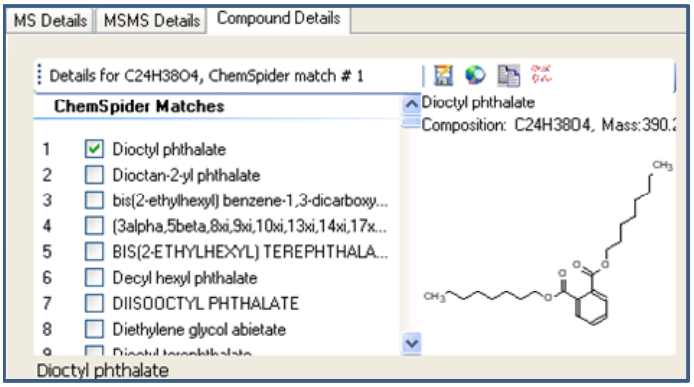


Figure 6: Showing database structure search results for chemical formula C24H38O4 as dioctyl phthalate, a plasticizer found in storage containers

Step 4: Correlate and Compare Impurity Trends across Multiple Batches

Results table for each individual batch can be correlated to see the trends and % levels of both related and unrelated impurities (Figure 8). Each impurity can also be corrected with a UV response factor to assign the most accurate % levels without under estimating or over estimating based on non-uniform MS response. The UV response factor is obtained by comparing MS and UV signal for impurities spiked at equal concentrations

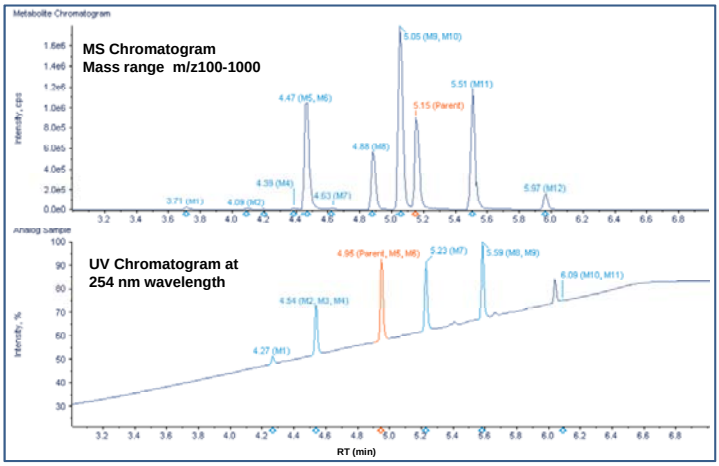


Figure 7: MS and UV chromatogram showing impurity response and RT correlation

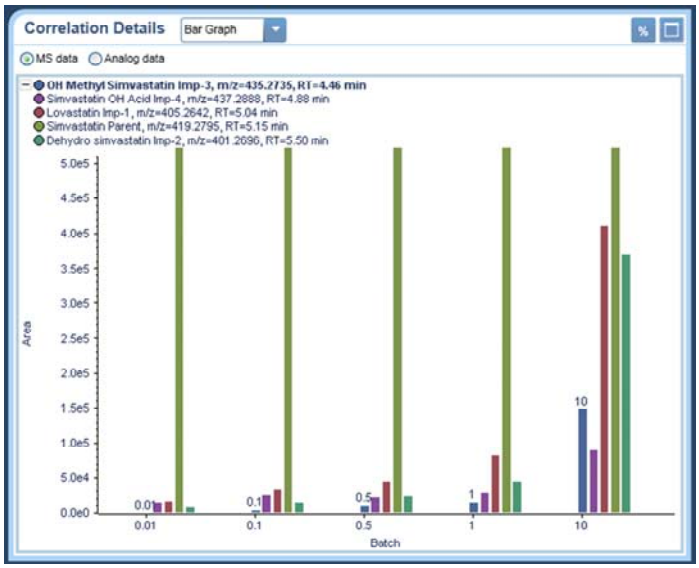
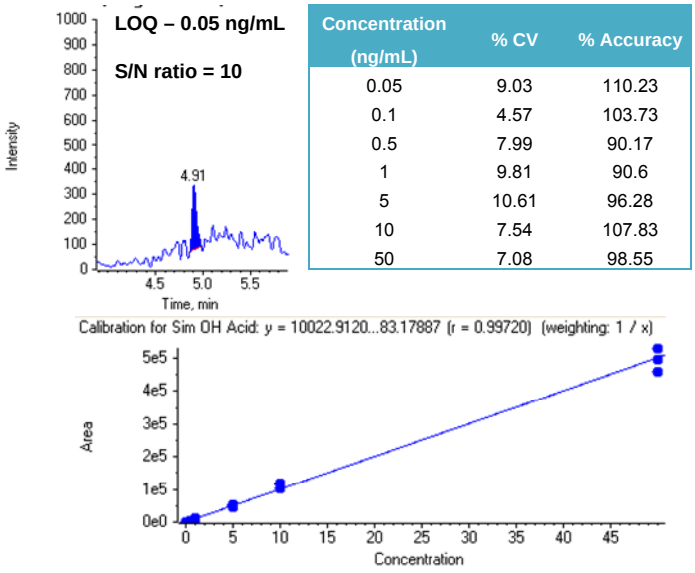


Figure 8: Correlate and compare simvastatin and its related impurities across five different batches

Correlation module with in MetabolitePilot software can also be used to follow the formation of a particular impurity like degradation product from photolysis or hydrolysis in case of accelerated stability studies or long term shelve life studies in bulk drug manufacturing and formulation production.

Step 5: Quantification of Related Impurities

Each individual impurity can be quantified using either internal calibration curve or external calibration curve. Absolute quantification of related impurities was achieved from the same analytical run that was used for identification and confirmation



Concentration (ng/mL)	% CV	% Accuracy
0.05	9.03	110.23
0.1	4.57	103.73
0.5	7.99	90.17
1	9.81	90.6
5	10.61	96.28
10	7.54	107.83
50	7.08	98.55

Figure 9: showing OH Methyl Simvastatin Imp-4 calibration curve, accuracy, precision and LOQ at 0.05 ng/mL with S/N -10

Compound name	LOD ng/mL	LOQ ng/mL	Precision %	Accuracy %
Lovastatin Imp-1	0.01	0.05-10	5.1	88-103
Dehydro Simvastatin Imp-2	0.01	0.05-10	7.5	98-102
OH Methyl Simvastatin Imp-3	0.01	0.05-50	4.8	87-99
Simvastatin OH Acid Imp-4	0.01	0.05-50	3.6	89-103
Dimer Imp-5	0.1	0.5-100	2.1	95-101

Table 2: Showing LOD, LOQ in ng/mL, % precision and % accuracy for all 5 simvastatin related impurities.

Calibration range, LOD, LOQ and statistics were evaluated for all other impurities related to simvastatin and summarized in table 2 as shown above. The sensitivity levels were sufficient enough to quantify impurities as low as 0.1%.

Conclusion:

The large number of compounds under investigation in drug discovery presents a significant analytical challenge for the detection, quantitation, and characterization of the compounds. The quantitative power of the TripleTOF family of instruments along with its advantages in speed, mass accuracy, and resolution presents unique returns in this space.

- A highly efficient single injection workflow was shown for the identification, characterization and quantification of Simvastatin and its impurities as low as 0.1%
- Fast scanning speed (100Hz), mass accuracy and triple quadrupole level sensitivity of TripleTOF™ 4600 system aided in identification and quantification at the same time
- Highly effective data processing algorithms, automated formula assignment, fragment interpretation and structure search capabilities helped in confirmation of impurities without much effort
- Correlation functionality with in MetabolitePilot software helped comparing impurity levels in multiple batches after UV response correction
- Simvastatin impurity profiling results were reported in detailed or summary format

References:

1. Guidance for Industry Q3A Impurities in New Drug Substances, June 2008, Revision 2.
2. U.S. Food and Drug Administration. Guidance for Industry, Q3B Impurities in New Drug Products. July 2006
3. Zhang H, Zhang D, Ray K, Zhu M. Mass defect filter technique and its applications to drug metabolite identification by high-resolution mass spectrometry. J Mass Spectrom. 2009 Jul;44(7):999-1016
4. Pilaniya K, Chandrawanshi HK, Pilaniya U, Manchandani P, Jain P, Singh N.Recent trends in the impurity profile of pharmaceuticals. J Adv Pharm Technol Res. 2010 Jul;1(3):302-10.
5. Sanjay B. Bari, Bharati R. Kadam, Yogini S. Jaiswal, Atul A. Shirkhedkar Impurity profile: Significance in Active Pharmaceutical Ingredient Eurasian Journal of Analytical Chemistry Volume 2, Number 1, 2007

Acknowledgements:

Authors would like to thank Steve Taylor, Yun-Yun Zhao, Kerong Zhang and Ping Du for their critical review.

Publication number: 09320114-01

Headquarters
353 Hatch Drive | Foster City CA 94404 USA
Phone 650-638-5800
www.absciex.com

International Locations
For our office locations please call the division headquarters or refer to our website at
www.absciex.com/offices