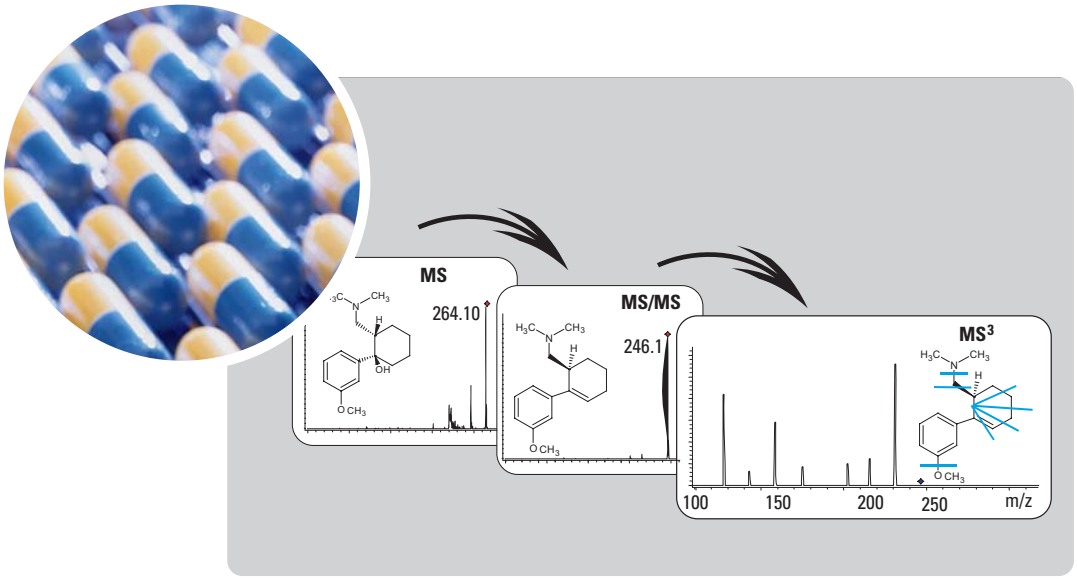
Impurity Profiling with the Agilent 1200 Series LC System

Part 1: Structure Elucidation of Impurities with LC/MS

Application Note

Edgar Nägele



Abstract

Today, it is necessary to identify and confirm the identity of all by-products appearing in the process of the development and manufacturing of a new drug substance in the pharmaceutical industry. In this Application Note the identification of by-products is demonstrated by structure elucidation by means of LC ion trap MS/MS and MS^3. In addition, the identity of the synthesis by-products will be confirmed by accurate mass measurement of the molecular ions by LC/ESI TOF. Subsequent parts of this series of Application Notes will show method development and validation of a QA/QC method to detect the identified impurities in the final dosage form of the drugs[3-6].

Agilent Technologies

Introduction

In modern pharmaceutical drug development and manufacturing it is crucial to identify minor impurities and by-products with the highest possible confidence because of their potential toxic effects on humans. The profiling of impurities in different phases of drug R&D is of extreme importance and a bottleneck in the entire process. Therefore, large efforts are made to develop strategies for fast impurity profiling using chromatographic, spectroscopic and hyphenated techiques[1]. In combination with the resolving power of liquid chromatography, ion trap instruments are widely used mass spectrometric tools for the structure elucidation by means of their MS/MS and MS^n capabilities. With these instruments, it is possible to break the molecular ion of the investigated substance in fragments, which are useful for the structure elucidation. Additionally, the mass spectrometric measurement of accurate molecular mass and consequently the calculation of the empirical formula is a common method for the identification and identity confirmation of an unknown compound. ESI orthogonal acceleration time-of-flight (oaTOF) MS instruments are capable of handling this task. This Application Note will discuss the identification of synthesis by-products and residual educts derived from a synthesis of a pharmaceutical drug[2] (figure 1) by ion trap and ESI oaTOF mass spectrometry.

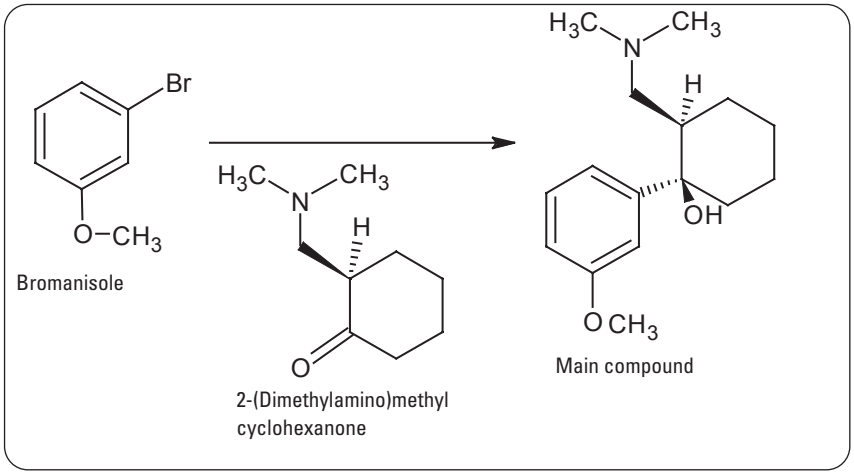


Figure 1
Synthesis of the pharmaceutical main target compound.

Experimental

Equipment

- Agilent 1200 Series Rapid Resolution LC system (RRLC): Agilent 1200 Series binary pump SL with degasser, Agilent 1200 Series high performance autosampler SL with thermostat, Agilent 1200 Series thermostatted column compartment, 1200 Series diode-array detector SL
- Agilent 6000 Series mass spectrometers: Agilent 6210 Series Time-of-Flight (TOF), Agilent 6330 Series Ion Trap
- Software: ChemStation B01.01, Ion Trap software 5.2 for instrument control with Data Analysis software 4.2. TOF software A.02.01 for instrument control and Analyst software for data analysis
- Columns: ZORBAX SB C18 2.1 x 150 mm, 1.8-µm particle size.

Method

- Solvent A: Water with 0.1% TFA
  Solvent B: AcN with 0.1 % TFA
- Flow: 0.5 mL/min
- Gradient 1: 0 min – 5 % B, 30 min 95 % B, 32 min 95 % B
- Gradient 2: 0 min – 5 % B, 30 min 50 % B, 31 min 95 % B, 32 min 95 %B. Stop time: 32 min, Post time: 10 min.
- DAD: 2-µL cell, 10-mm path, 270 nm ± 4 nm, ref. 360 +/-8 nm, width 0.1 min.
- Injector: 1-µL injection volume, needle wash 5 s with MeOH/Water 1/1
- Column oven: 60 °C
- MS – Ion Trap: source 200 °C, positive polarity, dry gas 10 L/min, nebulizer 40 psi, ICC 125000, autom. MS/MS and MS^3
- MS – TOF: source 200 °C, positive polarity, dry gas 12 L/min, nebulizer 40 psi, skimmer 40 V, scan m/z 100 – 1000, reference mass solution switched on

Results and discussion

To discover all possible impurities in a pharmaceutical drug compound during the development and manufacturing process it is very important to use several orthogonal separation techniques such as liquid chromatography, gas chromatography or thin-layer chromatography in several methodologies, for example, with different columns, solvents and other instrument parameters[1]. To detect all possible impurities in a technical drug sample, an LC as well as a GC separation was used as the initial analytical method. The LC analysis, by using a gradient with a high organic content (gradient 1) for the separation, resolved five impurities in a minor concentration of about 0.1% each (figure 2A). The comparison of the educts used in the synthesis with their retention time revealed impurity F as 3-bromanisole. To confirm the identity, the UV spectra were compared because these compounds did not produce a signal in the coupled electrospray mass spectrometer. The educt impurity E was neither detectable by UV nor ESI-MS. Therefore, the sample was analyzed by GC-FID and the compound could be detected and confirmed by retention time comparison (data not shown). To achieve better separation for the remaining impurities and their analysis by time-of-flight and ion trap mass spectrometry, a shallower gradient with up to 50 % organic solvent was used (gradient 2). With this method the remaining four impurities were sufficiently resolved for their mass spectrometric analysis to gain the necessary TOF-MS as well as ion trap MS/MS and MS^3 data for structure elucidation (figure 2B).

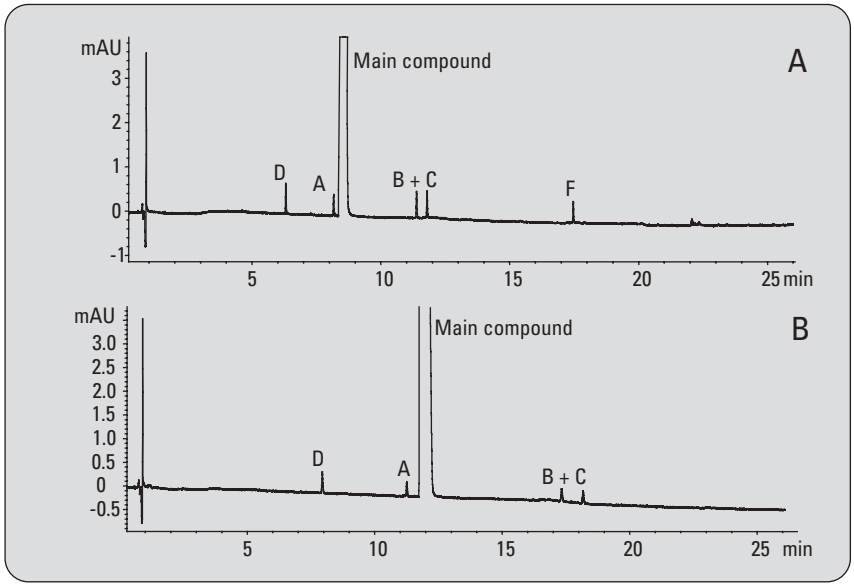


Figure 2
A) High resolution LC separation of the drug and impurities with gradient 1 and detection at UV 270 nm. B) High resolution LC separation of the drug and its impurities with gradient 2 and detection at UV 270 nm.

Impurity	Formula	Calculated mass	Measured mass	Mass accuracy [mDa]	Mass accuracy [ppm]
A	$C_{16}H_{26}NO_2$	264.1964	264.1957	-0.7	2.5
B	$C_{16}H_{24}NO$	246.1858	246.1850	-0.8	3.2
C	$C_{16}H_{24}NO$	246.1858	246.1851	-0.7	2.9
D	$C_{15}H_{24}NO_2$	250.1807	250.1804	-0.3	1.2

Table 1
Measurement of accurate masses by LC/TOF analysis of impurities and confirmation of the empirical formulas by calculating the relative mass errors.

To calculate empirical formulas for all impurities, an LC/MS-TOF analysis was performed to measure the accurate masses. It was possible to confirm all suggested formulas with sufficient mass accuracies in the single digit ppm range (table 1) with this experiment. The main compound itself has the empirical formula $C_{16}H_{26}NO_2$ with an accurate mass of 264.1964. The following empirical formulas for the detected impurities were calculated:

- Impurity A is an isomeric form of the main molecule, which has the same empirical formula $C_{16}H_{26}NO_2$ and a measured mass at m/z 264.1957.
- Impurity B has a measured mass at m/z 246.1850 and the calculated empirical formula $C_{16}H_{24}NO$.
- Impurity C has the same measured mass m/z 246.1851 as impurity B and they are isomeric forms of the same molecule.
- For the remaining impurity D, the measured mass was m/z 250.1804 with the calculated empirical formula $C_{15}H_{24}NO_2$.

To create more dedicated

structural information about the impurities, an ion trap mass spectrometric analysis was performed. The main peak at a retention time of 12 minutes was detected with its molecular ion at m/z 264.1 in the mass spectrum. The MS/MS of this molecular ion generated the fragment ion at m/z 246.1 due to a loss of a molecule of water. An MS^3 mass spectrum was not obtained because the fragment ion at m/z 246.1 did not undergo further fragmentation (figure 3). The diastereomeric impurity (A) was detected at a retention time of 11.3 minutes at the same m/z 264.1. The molecular ion of this compound also leads to the fragment ion at m/z 246.1 due to a loss of water in the MS/MS fragmentation (figure 4B). In contrast, an MS3 spectrum could be obtained in this case (figure 4C). The fragmentation of the ion at m/z 246.1 at the MS3 level generated the main ions at m/z 215.1 due to a loss of the methoxy group; the ion at m/z 202.1 due to a loss of the dimethyl amino group; and the fragment at m/z 121.1 due to a benzylic cation. The different fragmentation behavior of the main compound and its impurity A gives evidence of their diastereoisomerism because they are following different routes of water elimination on the MS/MS level, which leads to a stereochemically different ions at m/z 246.1. Fragmentation behavior similar to the main compound was found in impurity D. The molecular ion of impurity D,

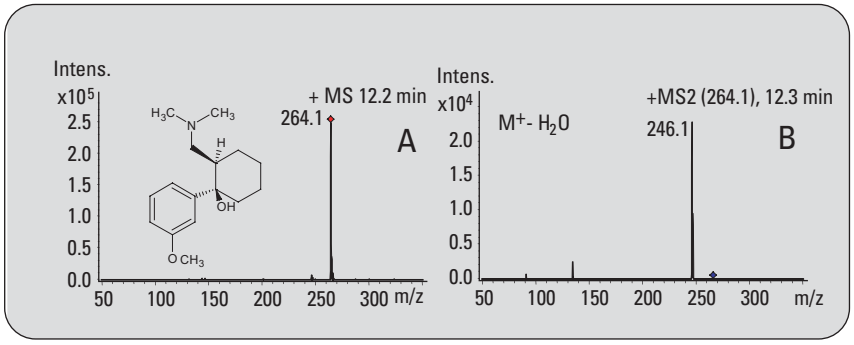


Figure 3
A) Ion Trap MS of the main compound with molecular ion at m/z 264.1 B) Ion trap MS/MS of the main compound molecular ion showing the ion at m/z 246.1.

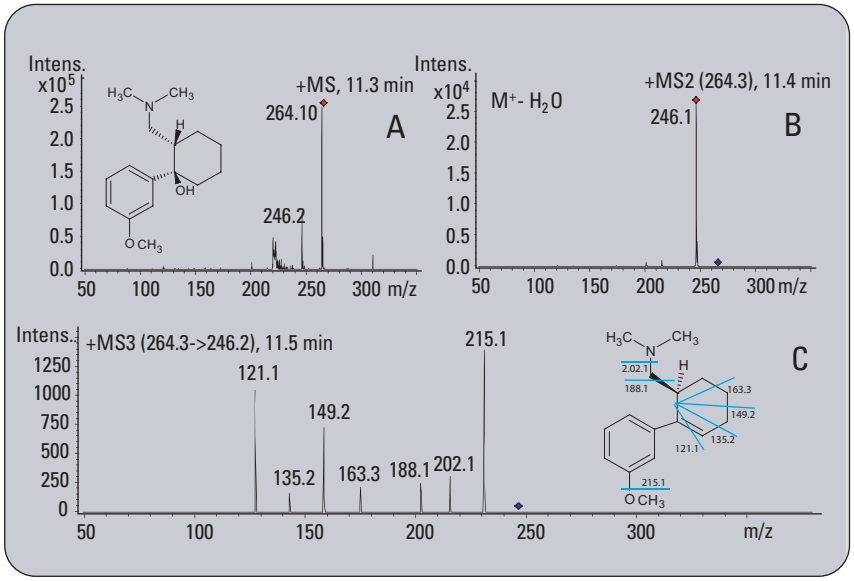


Figure 4
A) Ion Trap MS of impurity A with the molecular ion at m/z 264.1. B) MS/MS fragment ion of the impurity A at m/z 264.1. C) MS^3 fragmentation of the ion at m/z 246.1.

which is generated from the drug molecule by the loss of a methyl group at m/z 250.1, generates the fragment ion at m/z 232.1 during MS/MS fragmentation by a loss of water. An MS^3 fragmentation was not obtained (figure 5). Supported by similar fragmentation behavior as the main compound, it could be assumed that impurity D has the same stereochemistry as the drug molecule and therefore is the degradation product of a demethylation. The remaining impurities B and C were detected at m/z 246.2 at retention times of 17.3 and 18.2 minutes (figure 6A). The molecular ion of both impurities B and C correspond in two different ways to the molecular ion of the main compound due to a loss of water and the generation of a double bond in the molecule. The main peak in the MS/MS spectrum at m/z 202.2 is derived from a loss of the dimethyl amino group in both possible molecules (figure 6B). Further fragmentation in the MS3 experiment generated similar spectra for both impurities, resulting in fragments at m/z 173.9 and m/z 159.6 generated by a loss of the methoxy and methylene groups (figure 6C). With these results it was not possible to assign the correct location of the double bond in the molecules of the impurities B and C. Therefore, it is necessary to isolate and purify both compounds on a preparative scale for an additional NMR experiment to examine the correct stereochemistry[3].
The primary products of the syn-

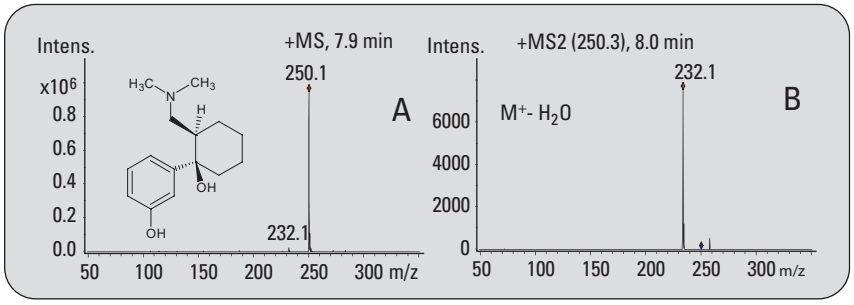


Figure 5
A) Ion trap MS of impurity D. B) Ion trap MS/MS fragmentation of m/z 250.1.

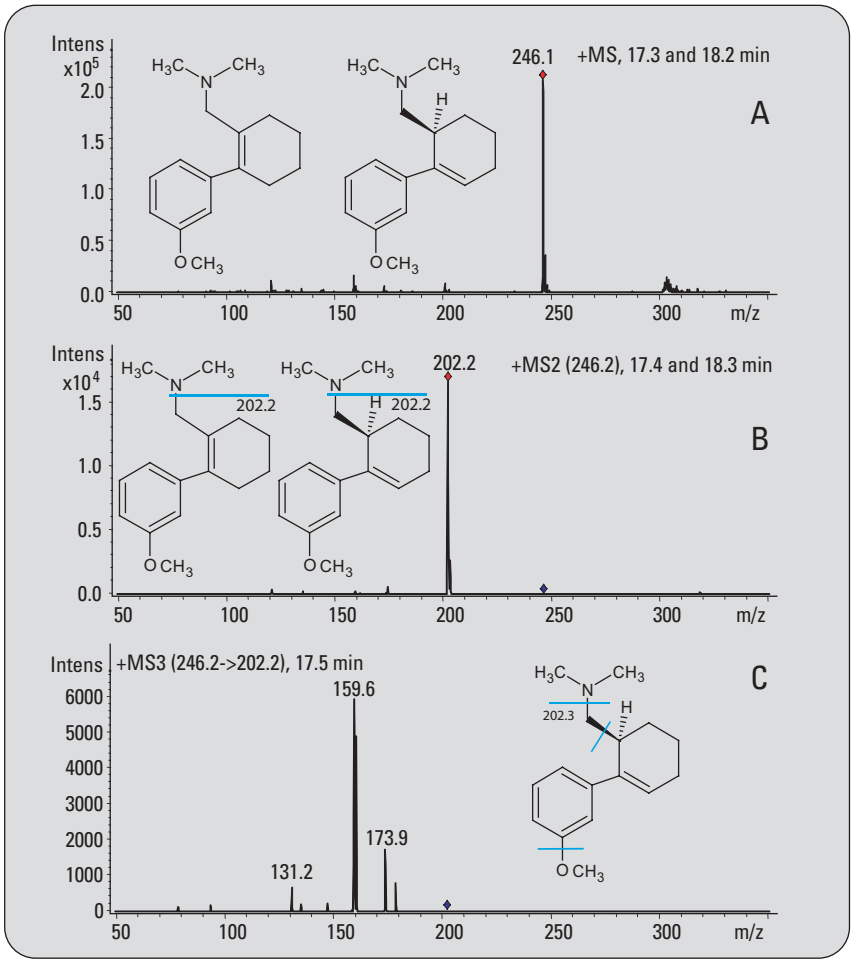


Figure 6
A) Ion Trap MS of impurities B and C at m/z 246.1. B) MS/MS fragmentation of impurity B and C to the fragments at m/z 202.2. C) Typical MS^3 fragmentation spectrum of the ions at m/z 202.2 from both impurities B and C.

thesis are the drug substance and its diastereomeric counterpart, which is found after a recrystallization step in the final product as the possible minor impurity A (figure 7). Beginning with the drug molecule itself, possible impurities are the degradation products, which are formed by a loss of water (impurites B and C), and the product of an undesired demethylation reaction (impurity D). Additionally, the educts of the synthesis are also possible by-products (impurities E and F).

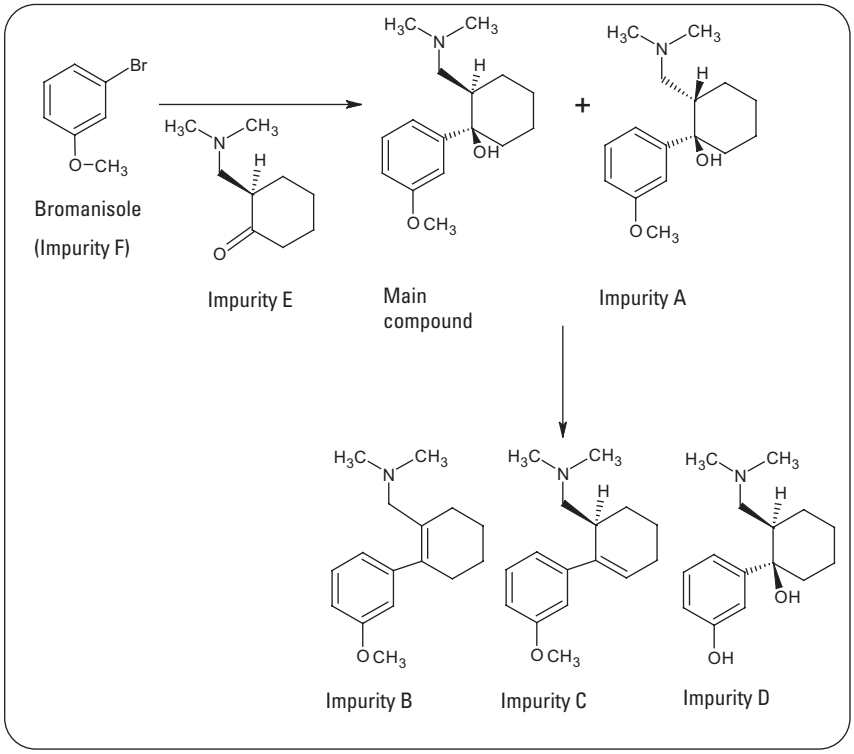


Figure 7
Identified impurities in the pharmaceutical drug synthesis of the main compound.

Conclusion

This Application Note demonstrates the use of the Agilent 1200 Series Rapid Resolution (RRLC) system with 1.8-µm RRHT columns in combination with the Agilent 6330 ion trap and the Agilent 6210 ESI TOF for the detection and structure elucidation of minor impurities in a pharmaceutical drug substance. With the 1200 Series RRLC system, the necessary resolution to detect all impurities was reached on 1.8-µm columns. The ion trap, with its MS/MS and MS^n capabilities, was used for structure elucidation and the ESI TOF for confirmation of the suggested formulas by accurate mass measurement. In this experiment all minor impurities were detected in a technical sample of a drug substance by means of orthogonal analytical methods (LC/UV, LC/MS and GC). For all impurities, structures were suggested based on the MS^n analysis and their identity was confirmed by accurate mass measurement and empirical formula calculation. Subsequent steps in the impurity profiling procedure include assigning undetermined impurities, which are purified on a preparative scale , for example, NMR anlysis[3]; and a method for QA/QC will be developed[4], valdidated[5] and applied[6].

References

1.
Sandor Görög "New Safe Medicines Faster: The Role of Analytical Chemistry." *Trends in Analytical Chemistry 22, 407.* **2003.**

2.
Victor A. Savelyev, Alexander G. Druganov, Elvira E. Shults, Genrikh A. Tolstikov, *Vorozhtsov Novosibirsk Institute of Organic Chemistry, Siberian Branch of the Russian Academy of Sciences 9 prosp. Akad. Lavrent'eva, 630090 Novosibirsk, Russia, http://www.nioch.nsc.ru/icnpas98/pdf/posters1/156.pdf*

3.
Udo Huber "Impurity Profiling with the Agilent 1200 Series LC System – Part 2: Isolation of Impurities with Preparative HPLC" *Agilent Application Note, publication number 5989-5618EN*, 2006.

4. Michael Frank "Impurity Profiling with the Agilent 1200 Series LC System – Part 3: Rapid Condition Scouting for Method Development", *Agilent Application Note, publication number 5989-5619EN*, **2006.**

5.
Angelika Gratzfeld-Huesgen "Impurity Profiling with the Agilent 1200 Series LC System, Part 4: Method Validation of a Fast Method" *Agilent Application Note, publication number 5989-5620 EN*, **2006.**

6.
Angelika Gratzfeld-Huesgen "Impurity Profiling with the Agilent 1200 Series LC System Part 5: QA/QC Application Example with Complete Sequencing" *Agilent Application Note, publication number 5989-5621EN*, **2006.**

Edgar Nägele is Application Chemist at Agilent Technologies, Waldbronn, Germany.

www.agilent.com/chem/1200rr

Published October 1, 2006
Publication Number 5989-5617EN

Agilent Technologies