

**Analysis of
Genotoxic Impurities
in Drug Substances
Using Fast Liquid
Chromatography
Coupled to a Triple
Quadrupole Mass
Spectrometer**

ASMS 2009

Siji Joseph, Agilent
Technologies India Pvt. Ltd.,
Bangalore-48

ThPI 226



Overview

Fast LC-MS/MS methodology for rapid quantification of genotoxic impurities, 4-chloroaniline in chlorhexidine and impurity-D in atenolol, with a wide linearity range (~5 orders) at sub ng/mL (ppb) detection limit has been achieved.

Introduction

Genotoxic impurities are of major concern in the pharmaceutical industry wherein obtaining higher sensitivity is the primary challenge for quantitation. Tandem mass spectrometry (QQQ) is the technique of choice for quantification of such impurities using multiple reaction monitoring (MRM) for sensitivity and selectivity. Coupling the QQQ with rapid resolution liquid chromatography using sub 2 microns particle size columns provides additional benefits such as increased chromatographic resolution and shorter analysis time for high-throughput applications. 4-chloroaniline is a degradant of chlorhexidine while impurity D is an alkyl chloro compound among eight specific impurities of atenolol (cardioselective beta blocker). 4-chloroaniline and impurity-D are reported genotoxins. In this study quantitation of these genotoxic impurities has been obtained at sub ng/mL levels.



Figure 1: Agilent 6410B LC/MS System

Experimental

Experimental conditions for 4-chloroaniline quantification

Agilent 6410B QQQ with MassHunter software

- Electrospray positive mode ionization
- MRM mode (transition m/z: 128 → 93)

Agilent 1200 RRLLC

- Mobile phase: 0.1% formic acid in water (aqueous) and 0.1% formic acid in methanol (organic)
- Column: Zorbax Eclipse Plus C18, 4.6 × 100 mm, 1.8µm with a flow rate of 700 µL/min
- Column temperature: maintained at 40°C
- Gradient: 50 to 70% of organic from 0 to 3 minutes, retained at same percentage for one minute

Experimental condition for impurity-D quantification

Agilent 6410B QQQ with MassHunter software

- Electrospray positive mode ionization
- MRM mode (transition m/z: 244 → 107)

Agilent 1200 RRLLC

- Mobile phase: 0.1% trifluoroacetic acid in water (aqueous) and 0.05% trifluoroacetic acid in methanol (organic)
- Column: Zorbax Eclipse Plus C18 4.6 × 50 mm, 1.8 µm with a flow rate of 1.5 mL/min
- Column temperature: maintained at 25°C
- Gradient:

Time (min)	% Organic
0	5
0.1	20
2.5	20
5.0	30
6.5	40
7.0	40
7.1	5

Table 1: Gradient used for atenolol experiment

Different concentrations of impurities were spiked in API to prepare samples for quantification. Eluent from the LC was introduced into a Agilent 6410B QQQ mass spectrometer (Figure 1). All ions were monitored at a dwell time of 200 ms/ion.

Results and Discussion

Quantification of 4-chloroaniline in chlorhexidine

4-chloroaniline was observed to elute at ~2.8 minutes while chlorhexidine eluted at ~3.5 minutes (Figure 2). The fragmentation pattern for 4-chloroaniline and chlorhexidine are shown in Figures 3a & 3b.

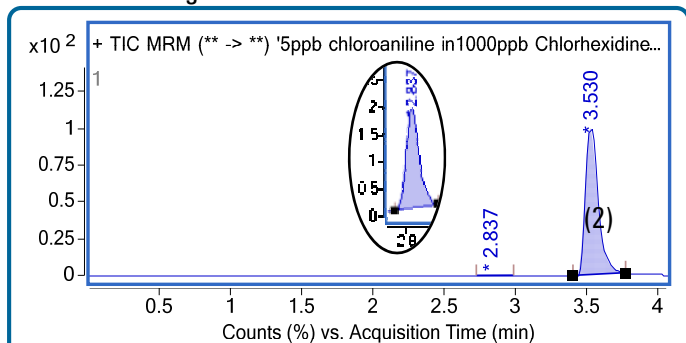


Figure 2: TIC of 0.5ppb 4-chloroaniline spiked in 1000ppb chlorhexidine

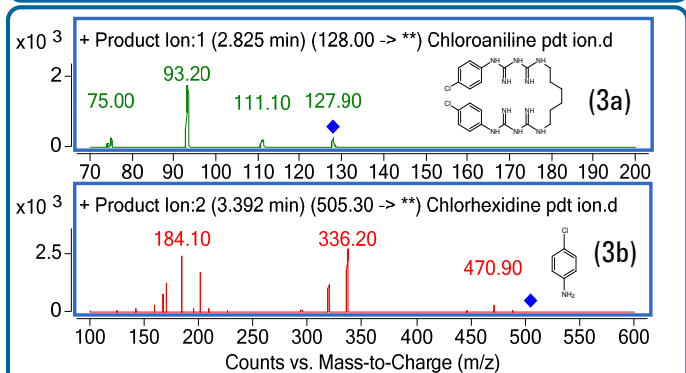


Figure 3: Product-ion pattern of 4-chloroaniline (3a) and chlorhexidine (3b).

Limit of detection obtained was 0.2ppb and linearity coefficient was $R^2 > 0.9998$ over a wide concentration range of 0.3 to 1000ng/mL (using 21 levels, 3 replicates) as shown in figure 4. Accuracy in measured concentration over the linearity range was found to be about $102.2 = 9.7\%$ which is well within the acceptable range.

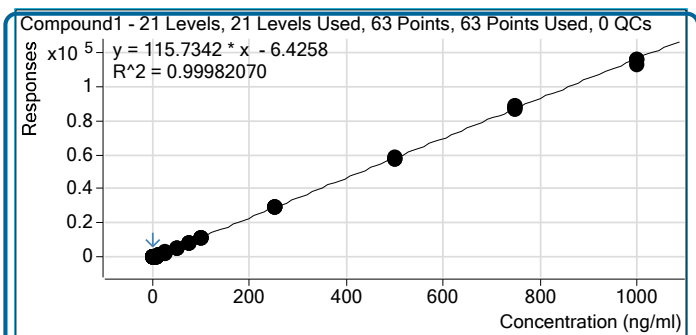


Figure 4: Linearity curve for 4-chloroaniline from 0.3 -1000 ng/mL

Results and Discussion

Quantification of impurity-D in atenolol

EP reports indicate atenolol has eight identified impurities named A to H wherein impurity F has two isomers. The chromatographic profile of these impurities as per EP and USP methods are shown in Figure 5 along with data obtained from the newly developed RRLLC method. The new RRLLC method has advantages like shorter run time, better resolution, enhanced sensitivity and improved separation of impurity F isomers.

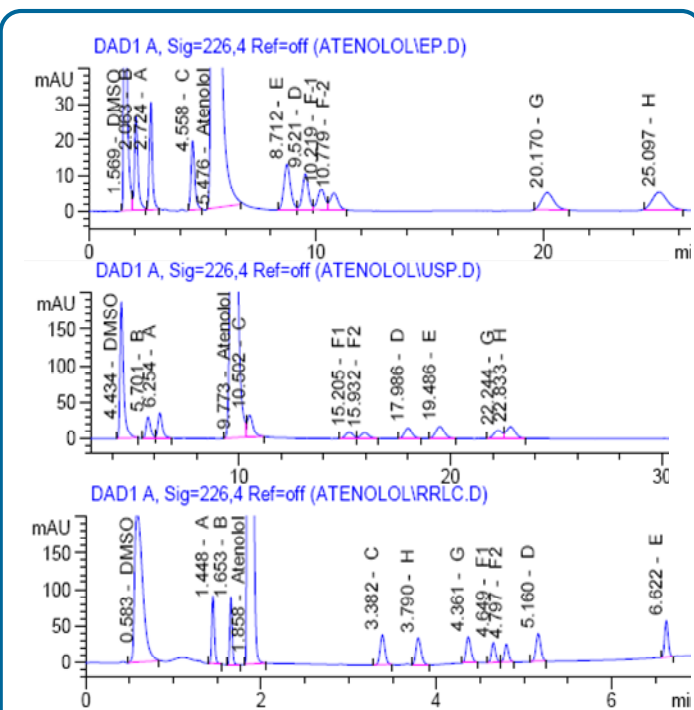


Figure 5: Chromatogram of Atenolol and impurities as per EP, USP and new RRLLC method

MS parameters were optimized using Agilent MassHunter Optimizer software which automatically optimized the data acquisition parameters for MRM (Multiple Reaction Mode) for each individual transition. It automates the selection of the best precursor ion, as well as optimization of fragmentor voltage and collision energy values for each transition. Table 2 shows the optimized parameters for atenolol as well as the impurities.

Results and Discussion

Compound	Precursor ion (m/z)	Product ion (m/z)	Fragmentor voltage (V)	Collision energy (V)
Impurity A	152.1	107	97	15
Impurity B	226.1	145	107	14
Atenolol	267.2	145	129	26
Impurity C	208.1	133	82	10
Impurity H	249.1	172	124	12
Impurity G	268.1	145.1	128	22
Impurity F	474.3	281.1	178	34
Impurity D	244.1	107	111	23
Impurity E	359.1	107	125	47

Table 2: Optimized MS parameters from MassHunter Optimizer

The fragmentation pattern for all peaks areas shown in Figure 6.

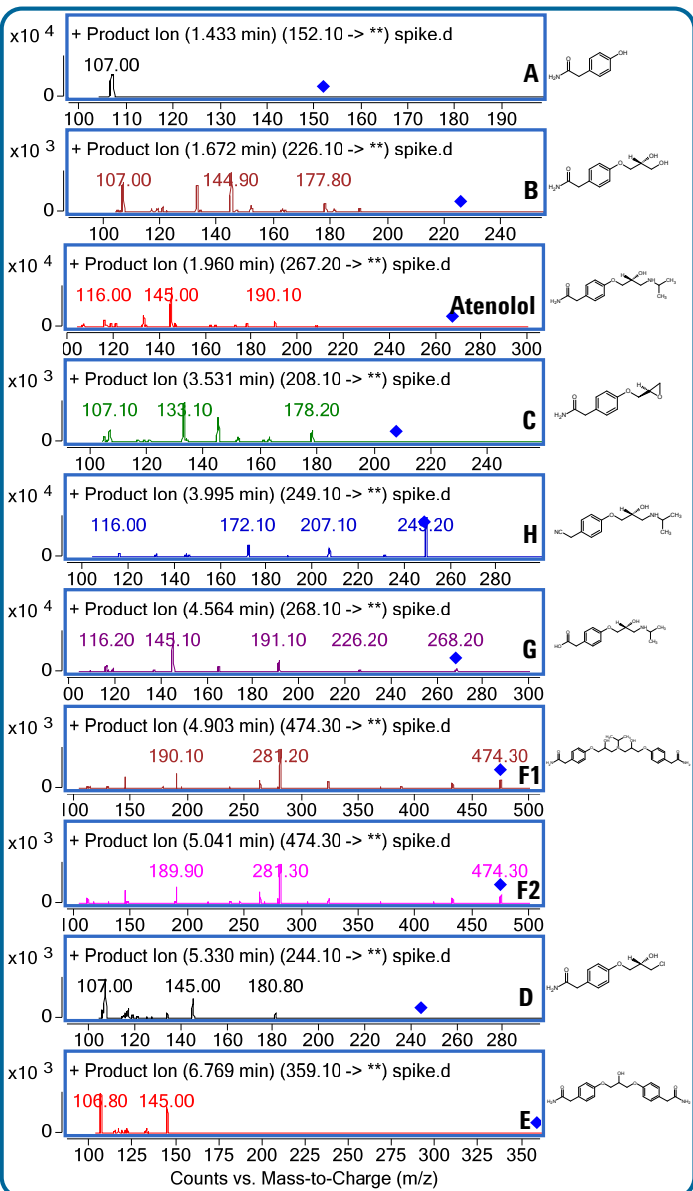


Figure 6: Product ion pattern of atenolol and impurities

Results and Discussion

Extracted ion chromatogram (EIC) of atenolol and impurities from MRM analysis are shown in Figure 7.

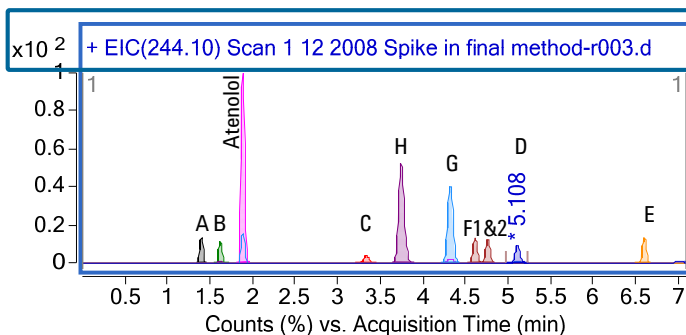


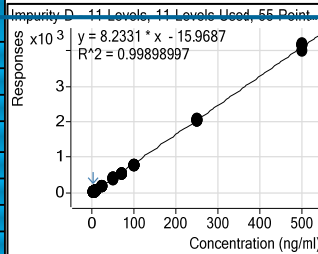
Figure 7: EIC of atenolol and impurities

Linearity curves were plotted with peak area versus concentration for all impurities from LOQ level to 500ppb. LOD, LOQ and linearity coefficient values are tabulated in Table 3, which shows an excellent linearity (R^2) >0.99 for all impurities. The linearity curve for impurity D is shown in Figure 8.

Sample	LOD (ppb)	LOQ (ppb)	Linearity
Impurity A	<1	1	>0.991
Impurity B	2	4	>0.999
Impurity C	2	3	>0.999
Impurity D	3	5	>0.999
Impurity E	2	3	>0.994
Impurity F	2	4	>0.999
Impurity G	<1	1	>0.995
Impurity H	1	2	>0.992

Table 3: LOD, LOQ and linearity coefficient values for all impurities

Figure 8: Linearity curve for impurity-D



Conclusions

- A fast, accurate, sensitive and reliable LC/MS/MS method has been developed for accurate measurement of genotoxic Impurities in atenolol and chlorhexidine.
- This method can be conveniently adopted for routine QA/QC analysis in pharmaceutical industry.