

## Quantitation and Identification of Pharmaceutical Compounds by HPLC/MS

### applying a Unique Combination ESI/EI Ion source

Today's demands for **qualitative und quantitative mass spectrometric applications** require continuous development of new technologies. **Bio and Life Sciences** act as driving engines pushing especially the development of modern hyphenated systems in GC/MS, LC/MS and CE/MS methodology.

In our application laboratory the **new and unique AMD Combination ESI/EI ion source** is applied to **HPLC/MS methodologies** in these fields and some typical results are described here.

Low limits of detection for quantitations in the order of **5–10 femtomol/μl** in real life samples are achieved.

Accurate mass determinations for substance identification are achieved with an **accuracy of better than 5 ppm** at a resolving power of  $R= 3000$  (10% valley) or  $R= 5000$  (FWHM).

The **unique AMD Combination ESI/EI ion source** is part for all AMD Intectra mass spectrometers and also may be attached to other mass spectrometers as an upgrade module

**The Innovators in Magnetic Sector Mass Spectrometry**

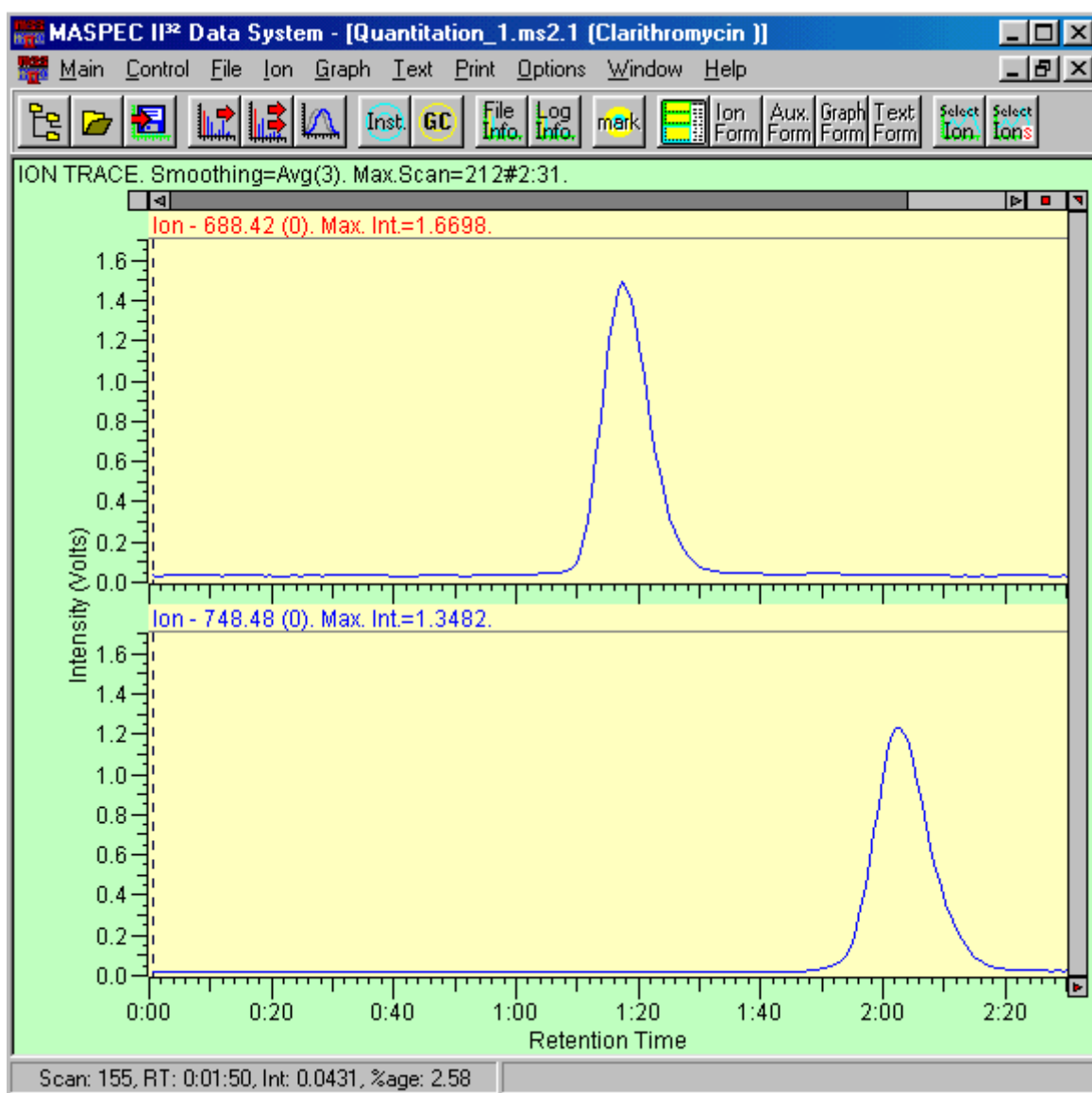
## Results

### A) Quantitation by Selected Ion Monitoring (SIM)

An HPLC/MS method has been developed for a rapid and automated quantitative analysis of **Clarithromycin** in human plasma samples as a basis for efficient pharmacokinetic studies. **Oleandomycin** has been used as an internal standard compound. We used the protonated molecular ions of both compounds for the ion chromatograms and subsequent quantitation.

### STANDARD SAMPLE

Ion chromatograms of **Oleandomycin ( $C_{35}H_{61}NO_{12} + H^+$ )** at  $m/z$  688.42 and **Clarithromycin ( $C_{38}H_{69}NO_{13} + H^+$ )** at  $m/z$  748.48



**Figure 1.** Ion chromatograms of a **standard sample** with a **concentration of 1 ng/μl** for both compounds which was used to evaluate the chromatographic conditions and to select the SIM parameters

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## PLASMA SAMPLE

Ion chromatograms of Clarithromycin ( $C_{38}H_{69}NO_{13} + H$ )<sup>+</sup> at m/z 748.48 for different concentrations

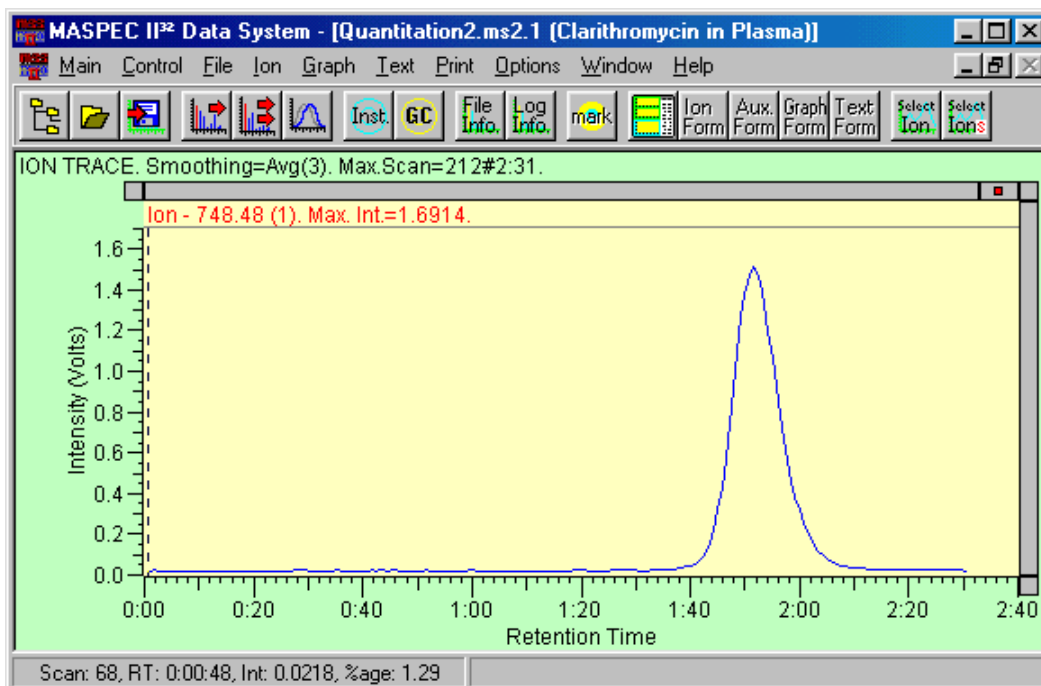


Figure 2a. Ion chromatogram at a concentration level of 1 ng/μl

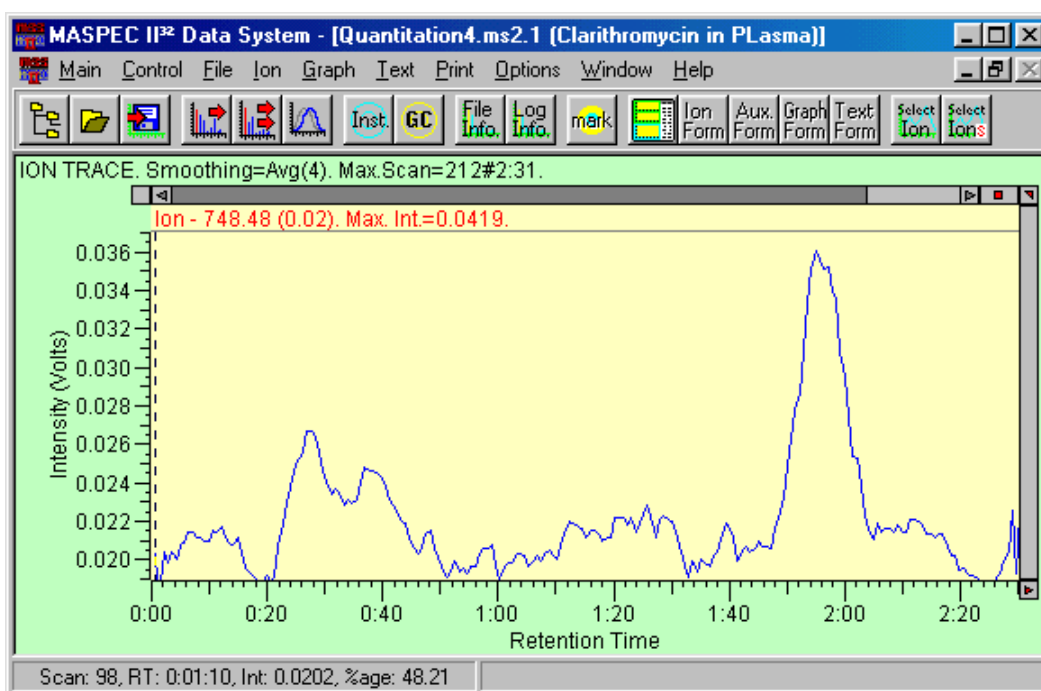


Figure 2b. Ion chromatogram at a concentration level of 10 pg/μl (13 femtomol/μl)

The results above indicate that in HPLC/MS-SIM mode a **detection limit** for quantitative analyses in real life samples can be obtained at concentration levels in the order of 5 – 10 femtomol/μl.

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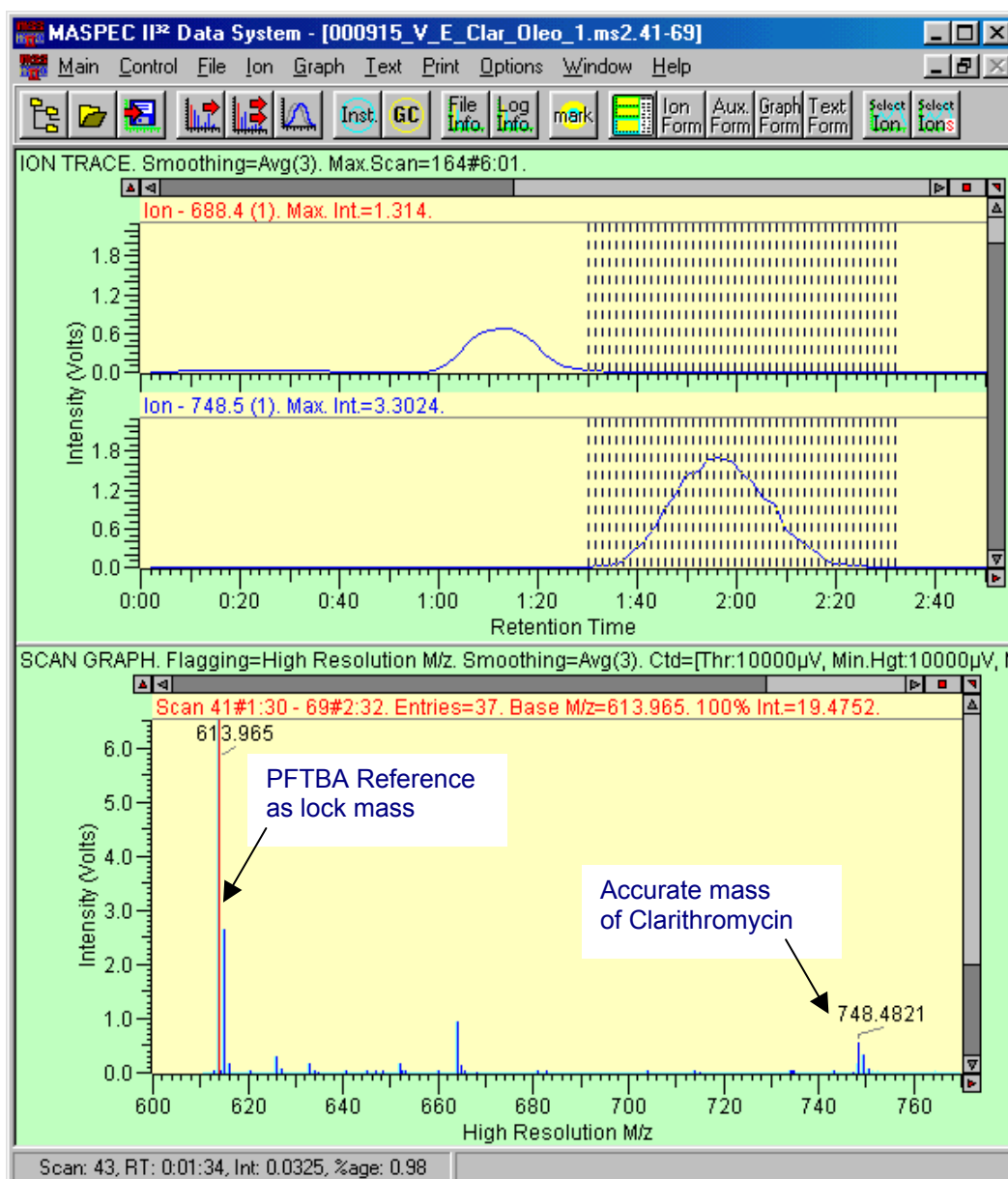
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## B) Substance Identification by Accurate Mass Determination

As described above for quantitative analyses in **HPLC/MS** mode the standard sample has been used at a concentration level of approx. 30 ng/ $\mu$ l to verify substance identification. For this purpose ions of the analyte have been produced by **Electrospray Ionisation** in the API region, at the same time ions of the reference compound have been produced by **Electron Impact Ionization**, independently without interferences of sample flows and ionization processes. The quality of the **double focussing AMD magnetic sector mass analyzer** and the **unique arrangement of the API/EI ion source** allows the **simultaneous recording of both ion species** formed in different ionization areas. As a result unequivocal accurate mass determinations using simple calibration and lock mass techniques can be achieved.

**Ion chromatograms of Oleandomycin (ESI) and Clarithromycin (ESI) and mass spectrum of Clarithromycin (ESI) and PFTBA (EI)**



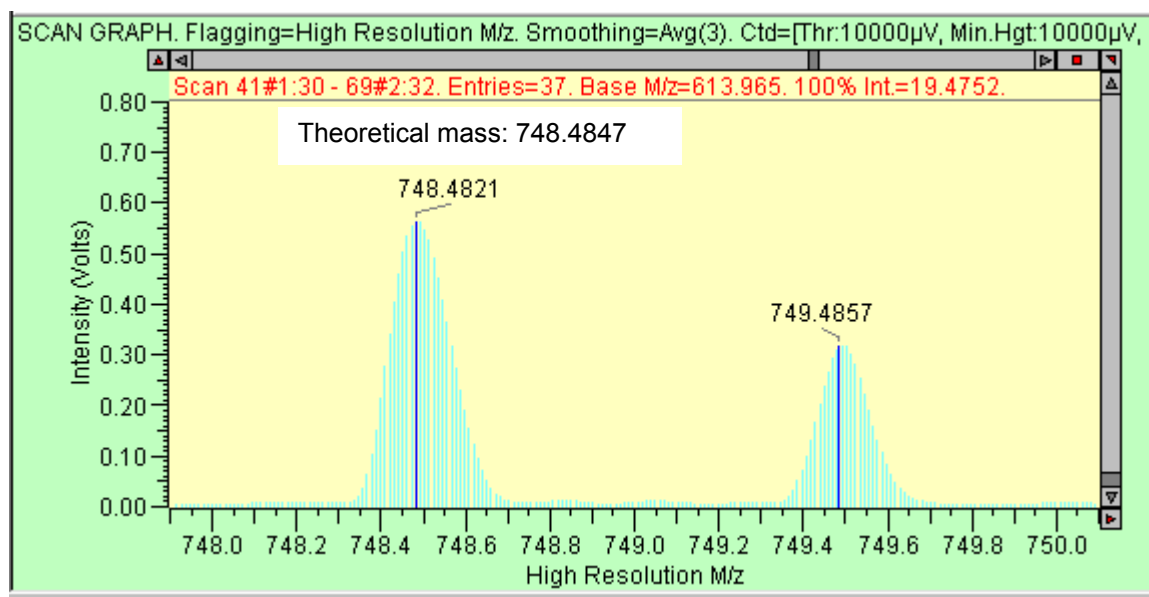
**Figure 3a.** Mass spectrum of **Clarithromycin** as analyte and **PFTBA** as reference compound in **combination ESI/EI** mode for accurate mass determination of the protonated analyte ion.

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## Accuracy of mass determination of the protonated molecular ion of Clarithromycin



**Figure 3b.** Quasi molecular ion group of **Clarithromycin** at a resolving power of 3000 (10% valley) or 5000 (FWHM) averaged during sample flow from HPLC. The accurate masses of the quasi molecular ion and the corresponding isotope have been determined with an **accuracy of better than 5 ppm**.

The results above indicate that the mass accuracy obtained in **HPLC/MS** mode is very well suitable for reliable substance identification in confirmational analyses.