AN IMPROVED METHOD FOR THE IDENTIFICATION AND QUANTIFICATION OF IMPURITIES IN ROXITROMYCIN SAMPLES USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET AND MASS DETECTION

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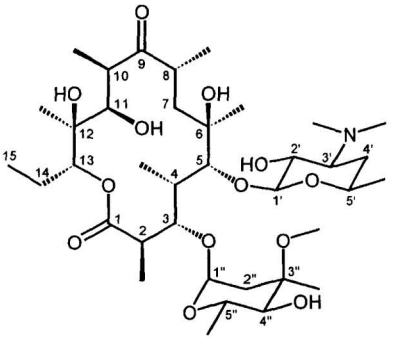
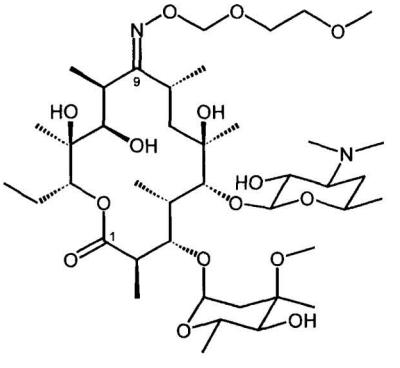
Erythromycin type macrolide antibiotics are widely used today as effective agents against infections with gram-positive bacteria, gram-negative cocci, mycoplasma and chlamydiae. One of the main disadvantage of erythromycin A (**1**), which has been the first clinically useful macrolide antibiotic, is the lability of this antibiotic natural product in acidic media. A successful concept to prevent this inactivation is the synthetic transformation of **1** to roxithromycin A (**2**) which is known to be acid stable and can be applicated orally.



erythromycin A (**1**)



roxithromycin A (**2**)

Though several articles have been published on the quantitative determination of impurities in macrolide antibiotics (see references in [2] and [3]), the analytical profiling of roxithromycin preparations is still a difficult question requiring an analytical method of high performance; first because the semi-synthetic antibiotic roxithromycin contains impurities deriving from both the fermentation process and the semi-synthetic transformation steps and secondly because nowadays analytical profiling of pharmaceutical drugs is recommended down to a range of 0.1%.

The European Pharmakopoeia [1] provides an HPLC method for the quality assurance of roxithromycin preparations, which does not allow an identification of impurity peaks by HPLC-MS, because a non volatile phosphate buffer system is used for elution. This prevents a valuation of roxithromycin samples, which have been obtained by new methods of preparation or purification and contain hitherto unknown impurities.

In this contribution we want to present a new HPLC method using a HyPURITY Elite C18 column for separation, a mixture of acetonitrile and aqueous ammonium acetate solution for elution and an UV detector adjusted to 215 nm for detection. Structural information about the by-products was obtained by comparison with reference substances and by HPLC-MS analysis giving mass spectrometric data of the molecule and fragment ions. A comparison of HPLC-MS and HPLC data revealed the complete information on the impurity profile of the analysed roxithromycin samples and allowed an assignment of mass spectrometric data to HPLC peaks as well as a correlation of peak areas to HPLC-MS peaks. With this new method well in hand we investigated crude and purified samples of industrial produced roxithromycin to demonstrate the impact of the final purification step on the impurity profiles.

		roxithromycin pure (RX-9100105)		roxithromycin crude (RX-9100199)	
assignment	m/e	R_t	% area	R_t	% area
erythromycin-Z-oxime	749.4			6.24	0.485
erythromycin A	734.5			7.03	0.094
erythromycin-E-oxime	749.4	7.72	0.152	7.77	0.457
roxithromycin C	823.5	10.01	0.181	10.20	0.656
	837.5			11.23	0.217
N-desmethyl-roxithromycin	823.5	12.48	0.284	12.61	0.362
roxithromycin A	837.5	15.42	98.357	15.45	92.914
roxithromycin A + second sidechain	925.5	19.04	0.104	18.23	0.716
roxithromycin A + second sidechain	925.5	22.30	0.093	22.45	0.381
roxithromycin B	821.5	26.65	0.479	26.86	1.408
roxithromycin A methylated	851.5	28.15	0.160	28.55	0.156
roxithromycin A chloralkylated (^{35}Cl : ^{37}Cl =3:1)	843.5 841.5	32.26	0.190	32.52	2.153
			100.000		99.999

References:

[1] Europäisches Arzneibuch, Nachtrag **2000**, Amtliche österreichische Ausgabe, Verlag Österreich, p. 1355-1357.

[2] S. Fochler, Diploma thesis, University of Vienna, **2001**.

[3] M. Stocker, Diploma thesis, University of Vienna, **2001**.