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Short Communication

Estimation and identification of non-polar compounds in clarithromycin bulk drug by high-performance liquid chromatography

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ABSTRACT

A high-performance liquid chromatographic method was developed to estimate and identify the non-polar related substances and process impurities that elute after N-demethyl-N-formyl-6-O-methylerythromycin A in clarithromycin bulk drug. This method separates at least fifteen compounds from the clarithromycin peak. All of the non-polar compounds can be detected at the 0.02% (w/w) level. Five bulk drug lots were examined for late-eluting compounds. The total related substances ranged from <0.10 to $<0.25\%$.

INTRODUCTION

Clarithromycin is a semi-synthetic broad spectrum antibiotic [1–10] being developed by Abbott Labs. and Taisho Pharmaceutical Co. Ltd. During the development of the synthetic route, a number of related substances or manufacturing impurities have been observed. Due to the varying polarity of these compounds, two separate methods were developed rather than using a gradient procedure. The purpose of this paper is to describe the method used for the estimation and identification of the non-polar related substances and process impurities that elute after N-demethyl-N--formyl-6-O-methylerythromycin A in clarithromycin bulk drug. Fig. 1 shows the structures for clarithromycin, IPCH oxime (related substance quantitation standard) and MeBHT (process impurity). Rationale for the indirect quantitative technique used in this procedure and the estimation and identification of the compounds that elute prior to N-demethyl-N-formyl-6-O-methylerythromycin is discussed elsewhere [11].

EXPERIMENTAL

Chemicals

Clarithromycin, IPCH oxime [6-O-methylerythromycin A-9-(1-isopropoxycy-

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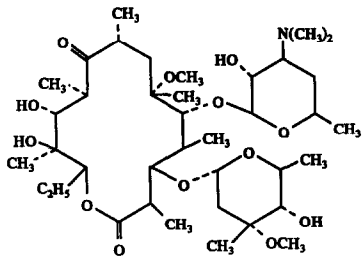
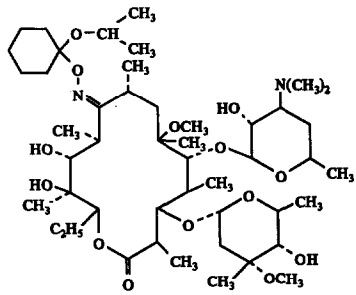
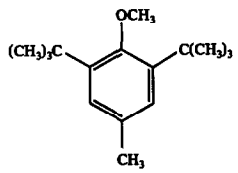


Clarithromycin



IPCH oxime

6-O-Methylerythromycin A-9-(1-isopropoxycyclohexyl) oxime (E)



MeBHT

4-Methoxy-3,5-di-tertbutyltoluene

Fig. 1. Structures for clarithromycin, IPCH oxime and MeBHT.

clohexyl)oxime (*E*)] and MeBHT [4-methoxy-3,5-di-*tert.*-butyltoluene] were synthesized. Acetonitrile and monobasic potassium phosphate (Fisher Scientific, Fair Lawn, NJ, U.S.A.) and phosphoric acid (J. T. Baker, Phillipsburg, NJ, U.S.A.) were HPLC and ACS grade, respectively. Distilled water was used without additional purification.

Apparatus and conditions

A modular chromatograph consisting of a pump (Spectra-Physics Model 8800), an autosampler capable of injecting 50 μl (Spectra-Physics Model 8780), a variable-wavelength ultraviolet detector capable of monitoring at 205 nm (Applied Biosystems Model 757), a oven capable of maintaining the column at 50°C (Anspec Model SM900) and a computing integrator to display the chromatogram and measure the area of the peaks obtained (Spectra-Physics Model 4200). A 150 × 4.6 mm I.D. column packed with Nucleosil C_{18} (100 Å, 5 μm) was used with a flow-rate of 1.0 ml/min.

Dilution solution and mobile phase

The dilution solution was prepared by mixing 1950 ml of acetonitrile and 1050 ml of 0.033 *M* monobasic potassium phosphate. The mobile phase was prepared by adjusting the pH of 2000 ml of dilution solution to 4.1 ± 0.1 with phosphoric acid. Prior to using, the mobile phase was filtered through a 0.45-μm nylon membrane and helium degassed. In addition to the mobile phase, the dilution solution was used as the solvent for all of the standard and sample preparations.

Solutions

For quantitation, a 1 μg/ml solution of IPCH oxime was prepared. For system suitability, a clarithromycin–ECH oxime (see Table I)–IPCH oxime solution corresponding to 20 mg/ml, 5 μg/ml and 8 μg/ml, respectively, was prepared. For identification of MeBHT, a 1 μg/ml solution was prepared.

System suitability

The system was acceptable for use when all of the following conditions were met: (1) the efficiency of the column for the IPCH oxime peak was ≥25 000 plates/m, (2) the resolution between the ECH and IPCH oxime peaks was ≥2.6 and (3) the relative standard deviation of two injections of the 1 μg/ml IPCH oxime solution was ≤3%. The clarithromycin–EPCH oxime–IPCH oxime solution was used for the column efficiency and resolution determinations.

Sample preparation

Transfer approximately 105 mg of accurately weighed clarithromycin bulk drug into a 50-ml volumetric flask. Dissolve and dilute to volume with dilution solution.

Assay procedure

Inject the sample preparation in duplicate. The 1 μg/ml IPCH oxime solution should be injected after every four to six samples to verify system response. Calculate the relative retention time (*RRT*) of all compounds that elute after N-demethyl-N-formyl-6-O-methylerythromycin A. From the data in Table I, identify all known compounds and determine the concentration (%, w/w) of each using: $[100/NRF(A_u/A_s)(C_s/C_u)]$, where A_u is the area of the individual related compound peak, A_s is the

TABLE I

POTENTIAL NON-POLAR COMPOUNDS

Compound	*RRT*	Abbreviation
(1) N-Demethyl-N-formyl-6-O-methylerythromycin	0.26	N-Formyl
(2) Erythromycin A-9-(1-isopropoxycyclohexyl)oxime (*E*)	0.51	EMIPCH oxime
(3) 6-O-Methylerythromycin A-9-(1-methoxycyclohexyl)oxime (*E*)	0.56	MCH oxime
(4) 1,1-Diisopropoxycyclohexane	0.76	Ketal
(5) 6-O-Methylerythromycin A-9-(1-ethoxycyclohexyl)oxime (*E*)	0.80	ECH oxime
(6) 6-O-Methylerythromycin A-9-(1-isopropoxycyclohexyl)oxime (*E*)	1.00	IPCH oxime
(7) 2'-Deoxy-3'-de(dimethylamino)-2',3'-epoxy-6-O-methylerythromycin A	1.06	Epoxy
(8) 4-Methoxy-3,5-di-*tert.*-butyltoluene	2.75	MeBHT

area of the IPCH oxime standard solution, C_u is the concentration of clarithromycin in the assay solution and C_s is the concentration of the IPCH oxime standard solution. For any unidentified compound estimate the concentration using a normalized response factor (*NRF*) of 1.00

RESULTS AND DISCUSSION

The octadecyl silane columns used for estimation of the polar related substances (YMC, A303, 250 × 4.6 mm I.D.) and the determination of clarithromycin potency (Nucleosil, 150 × 4.6 mm I.D.) were examined for the non-polar compounds. Increasing the acetonitrile concentration and lowering the pH of the of the mobile phase shortened the retention times and sharpened the peak shape for the compounds of interest. Both columns gave similar separation of the non-polar compounds. The Nucleosil column was chosen for the method because of its shorter analysis time and lower cost. A typical chromatogram for the analysis of non-polar compounds in clarithromycin bulk drug is found in Fig. 2.

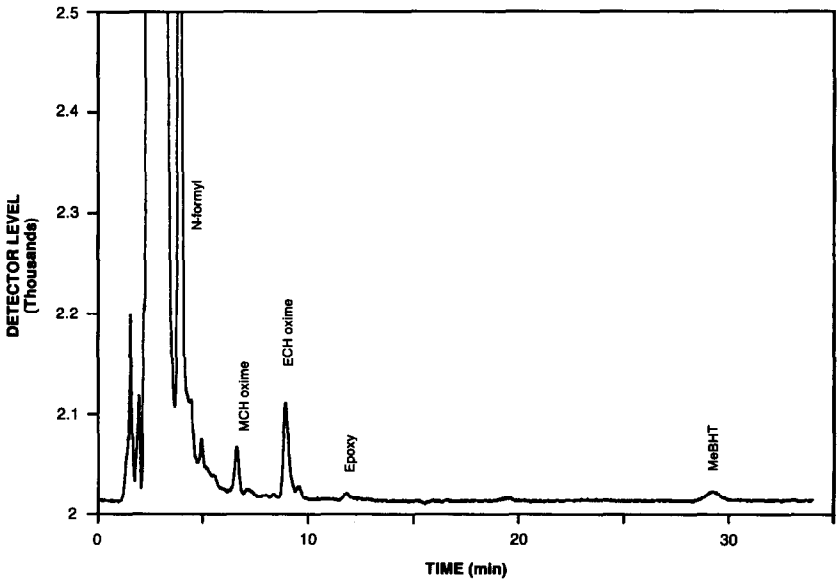


Fig. 2. Typical chromatogram of non-polar compounds observed in Clarithromycin. Refer to Table I for identification of compounds. Chromatographic conditions as stated in text.

The effects of pH and acetonitrile content on the elution characteristics of the related substance IPCH oxime and the highly retained process impurity MeBHT are shown in Fig. 3. This figure shows that slight changes in pH have minimal effect on the retention of the compounds examined. It also shows that slight changes in acetonitrile content cause retention times to vary significantly. Thus, MeBHT needs to be chromatographed daily so that it can be accurately identified.

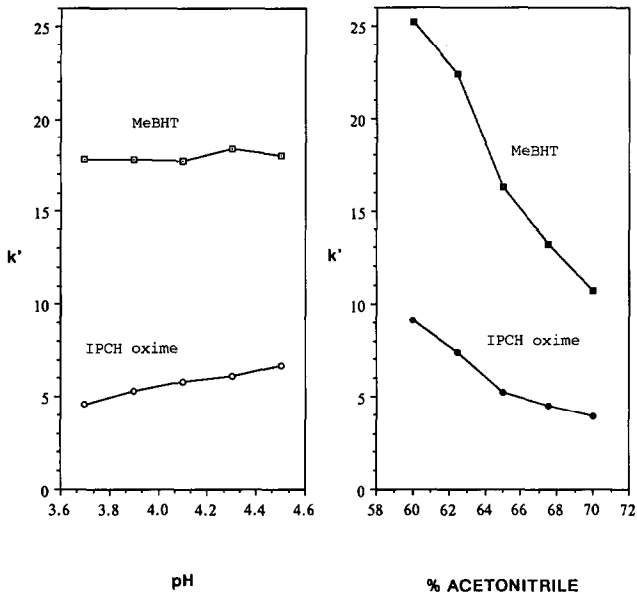


Fig. 3. Effect of pH and acetonitrile content on IPCH oxime and MeBHT capacity factors. Mobile phase for pH experiment: dilution solution was adjusted to specified pH with phosphoric acid. Mobile phase for acetonitrile experiment: acetonitrile–0.33 *M* phosphate solutions adjusted to pH 4.1 with phosphoric acid. Other chromatographic conditions as stated in text.

In the mobile phase scouting experiments, IPCH oxime solutions degraded when they were stored overnight in mobile phase. When solutions were stored in a mixture of acetonitrile–0.033 *M* phosphate solution (65:35), the IPCH oxime did not degrade. Table II shows that solutions containing approximately 0.5 mg/ml of IPCH oxime are stable for at least two weeks when stored under refrigerated conditions and for at least seven weeks when frozen.

Linearity of the detector response was demonstrated for IPCH oxime concentrations of 0.2 to 10.2 μg/ml. The plot of concentration *versus* peak area essentially passed through the origin and had a correlation coefficient of 0.9999. Using

TABLE II

STABILITY OF IPCH OXIME SOLUTION

Storage condition	Days	Taken (mg)	Found (mg)	Recovery (%)
Room temperature	3	27.76	25.08	90.3
Refrigerated	3	10.09	9.97	98.8
	8	10.09	9.89	98.0
	14	10.09	10.08	99.9
Frozen	14	12.27	12.28	100.1
	34	105.51	104.24	98.8
	51	108.87	106.64	98.0

TABLE III

RECOVERY OF IPCH OXIME ADDED TO CLARITHROMYCIN REFERENCE STANDARD

Day	Added (μg)	Found (μg)	Recovery (%)
1	2.02	2.06	102.0
	10.11	11.08	109.6
	20.21	22.85	113.1
2	2.04	2.06	101.0
	10.21	11.21	109.8
	20.41	21.98	107.7

a signal-to-noise ratio of 3:1, the limit of detection for the majority of the identified non-polar compounds is 0.2 μg/ml or 0.02% (w/w).

In this chromatographic system, some of the related substances that elute prior to clarithromycin are co-eluted in void volume. Thus, peak area percent calculations for the non-polar compounds are biased on the high side. Weight/weight percent data based on indirect quantitation provides a more accurate means of estimating the levels of related substances and process impurities in clarithromycin.

Since all of the identified related substances are macrolides and therefore, have similar if not identical molar absorptivities, a direct comparison to IPCH oxime can be used for estimation of these compounds. For unidentified compounds, a direct comparison to IPCH oxime is also used. For known process impurities, where the molar absorptivities are different, normalized response factors are required for accurate data. The *NRF* for MeBHT is 21.6.

TABLE IV

PRECISION DATA FOR NON-POLAR COMPOUNDS OBSERVED IN A LOT OF CLARITHROMYCIN

See Table I for identification of compounds. – = No peak.

RRT	Compound	Day 1[a]	Day 2[a]	Day 3[a]	Day 4[b]	Day 5[b]	Mean	SD
0.30		0.03	–	<0.02	<0.02	<0.03	0.02	–
0.31		0.04	–	–	–	–	<0.02	–
0.34		0.02	0.02	<0.02	<0.02	<0.02	<0.02	–
0.54	MCH oxime	0.02	0.02	0.02	0.02	0.02	0.02	0.00
0.65		–	<0.02	–	–	–	<0.02	–
0.72		–	<0.02	–	–	–	<0.02	–
0.80	ECH oxime	0.05	0.05	0.05	0.04	0.06	0.05	0.01
1.00	IPCH oxime	–	<0.02	<0.02	–	–	<0.02	–
1.12	Epoxy	<0.02	–	<0.02	<0.02	<0.02	<0.02	–
1.57		–	<0.02	–	–	–	<0.02	–
2.75	MeBHT	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	0.00

[a] Analyst 1.
[b] Analyst 2.

TABLE V

PERCENT NON-POLAR COMPOUNDS OBSERVED IN FIVE LOTS OF CLARITHROMYCIN

See Table I for identification of compounds. – = No peak.

RRT	Compound	Lot A (%)	Lot B (%)	Lot C (%)	Lot D (%)	Lot E (%)
0.30		<0.02	–	–	–	–
0.31		<0.02	–	–	–	–
0.34		<0.02	0.02	<0.02	–	–
0.51	EMIPCH oxime	–	<0.02	–	–	–
0.54		–	–	–	–	<0.02
0.56	MCH oxime	0.02	<0.02	<0.02	0.03	0.02
0.65		<0.02	–	–	<0.02	<0.02
0.72		<0.02	–	–	–	–
0.76	Ketal	–	–	–	<0.02	<0.02
0.80	ECH oxime	0.05	–	0.02	0.03	0.02
1.00	IPCH oxime	<0.02	–	–	–	–
1.06	Epoxy	<0.02	0.03	0.02	–	–
1.25		–	–	–	<0.02	<0.02
1.57		<0.02	–	–	–	–
2.75	MeBHT	<0.02	<0.02	<0.02	–	–
Total		<0.25%	<0.11%	<0.10%	<0.12%	<0.12%

On two separate days, IPCH oxime was added to clarithromycin reference standard at the 0.1, 0.5, and 1.0% levels. The data summarized in Table III show that acceptable recovery was obtained.

A sample of clarithromycin bulk drug was analyzed on five separate days. The analyses were performed on two different chromatographic systems by two analysts. The data summarized in Table IV show that the method is reproduceable.

Five lots of clarithromycin bulk drug were analyzed by this procedure. The data summarized in Table V show that the non-polar related substances in these lots ranged from <0.10 to <0.25%.

The results obtained in this work show that a rugged, sensitive, indirect quantitative high-performance liquid chromatographic procedure has been developed to estimate and identify potential non-polar compounds formed during the production of clarithromycin.

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