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Nadolol: high-pressure liquid chromatographic methods for assay, racemate composition and related compounds*

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Abstract: High-pressure liquid chromatographic (HPLC) methods have been developed for the determination of drug content, racemate A and related compounds in nadolol raw materials. The method for drug content and related substances resolved seven related compounds and several unknown impurities from the drug. The minimum quantifiable levels were 0.05% or less for four of the seven related compounds and 0.3% or less for the remainder. Total impurities in eight raw material samples ranged from 0.06 to 0.96% and assay levels ranged from 98.7 to 101.0%. The method was adapted for the determination of nadolol racemate A by a change in mobile phase composition. One raw material sample contained less than 40% of racemate A. Two samples which had a granular appearance were variable in racemate A content. The methods were adapted for the determination of drug and racemate A in nadolol tablets. Drug content in three tablet samples was between 96.2 and 98.4% and racemate A content was about 52%.

Keywords: *Nadolol; β-blocker; assay; impurities; racemates; HPLC.*

Introduction

Nadolol (I) is described by the USP [1] as a 1:1 mixture of racemates A and B, depicted as compounds IV and V, respectively, in Fig. 1. The two ring hydroxyl groups are in the *cis* configuration. Thus, although there are three asymmetric centres, only two pairs of enantiomers of the drug are possible. Racemate B is three times more potent as a β-blocker and antiarrhythmic agent than racemate A [2].

Structures of nadolol and several related compounds, selected for method development on the basis of synthetic routes [3–6] and availability, are presented in Fig. 1. Compounds VII and IX may be used as starting materials; III, VIII and IX are possible synthetic intermediates; II may be an immediate precursor; VI is a side product of the synthesis, and X is formed when VIII reacts with epichlorohydrin. Nadolol is stable to long exposures of normal levels of heat and light [5].

Methods for the assay of nadolol raw material and tablets based on gas chromatography [7], HPLC [5], titration [8], colorimetry [9] and NMR [10] have been described in the literature. The separation of nadolol diastereomers by reversed-phase HPLC has also been reported [11].

The United States Pharmacopeia monograph [1] for nadolol raw material sets rubric limits of 98.0–101.5% determined by non-aqueous titration and limits of 40–60% for racemate A, determined by infrared spectroscopy. Chromatographic purity is determined by TLC with quantitation by UV after scraping impurity bands off the plate. Tablet assay is by HPLC. There are no monographs for nadolol in the British Pharmacopoeia [12].

Experimental

Chemicals

Acetonitrile and methanol (Baker, Phillipsburg, NJ, USA), glacial acetic acid (Caledon Laboratories, Georgetown, ON, Canada) and sodium acetate (Fisher Scientific, Fairlawn, NJ, USA), were HPLC grade. Distilled water was deionized in a Sybron/Barnstead system. Compounds I–III and V–IX were obtained from E.R. Squibb (Princeton, NJ, USA). The mass, infrared and nuclear magnetic resonance spectra of these compounds were consistent with their respective structures. II contains a

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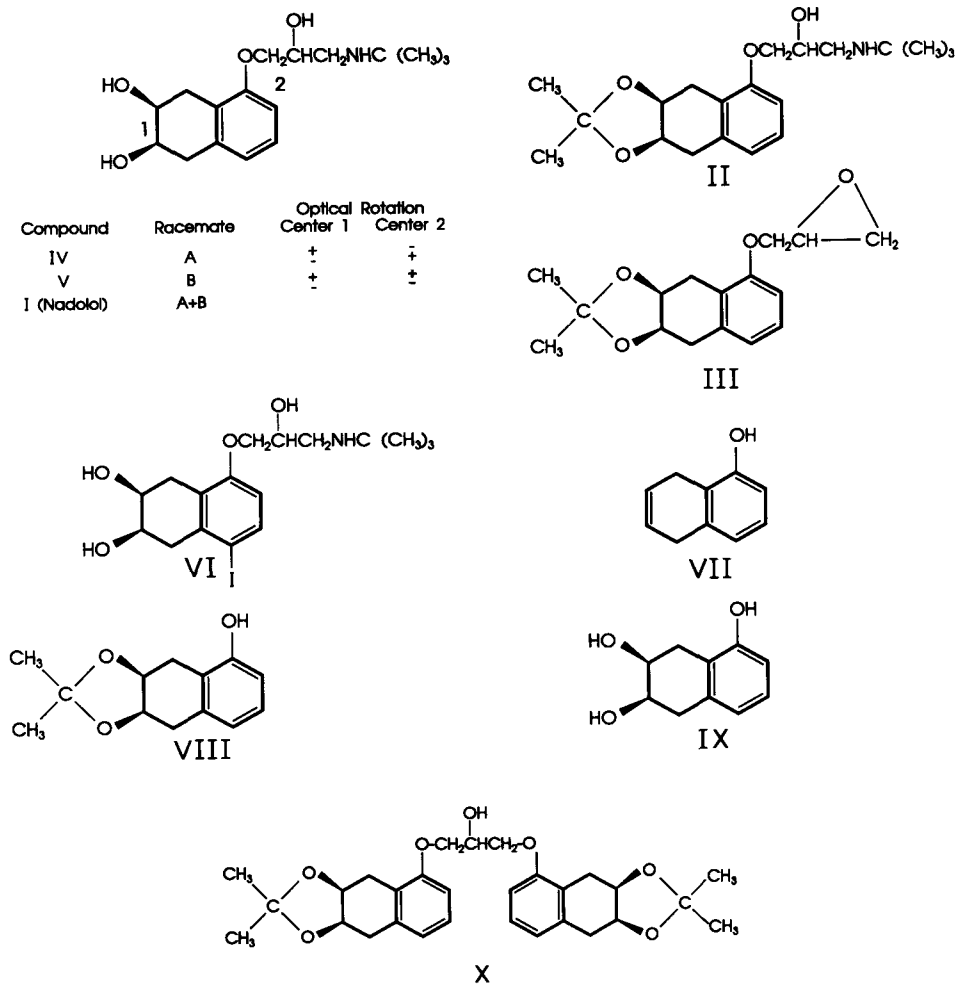

Figure 1
Chemical structures of nadolol and related compounds. I — Nadolol: 2,3-*cis*-1,2,3,4-tetrahydro-5-(2-hydroxy-3-(*tert*-butylamino)propoxy)-2,3-naphthalenediol; II — acetonide of 2,3-*cis*-1,2,3,4-tetrahydro-5-(2-hydroxy-3-(*tert*-butylamino)propoxy)-2,3-naphthalenediol; III — acetonide of 2,3-*cis*-1,2,3,4-tetrahydro-5-(2,3-epoxypropoxy)-2,3-naphthalenediol; IV — racemate A: 5-[*d*-3-(*tert*-butylamino)-2-hydroxypropoxy]-1,2,3,4-tetrahydro-*l*-*cis*-2,3-naphthalenediol, and 5-[*l*-3-(*tert*-butylamino)-2-hydroxypropoxy]-1,2,3,4-tetrahydro-*d*-*cis*-2,3-naphthalenediol; V — racemate B: 5-[*d*-3-(*tert*-butylamino)-2-hydroxypropoxy]-1,2,3,4-tetrahydro-*d*-*cis*-2,3-naphthalenediol, and 5-[*l*-3-(*tert*-butylamino)-2-hydroxypropoxy]-1,2,3,4-tetrahydro-*l*-*cis*-2,3,naphthalenediol; VI — 8-iodo-2,3-*cis*-1,2,3,4-tetrahydro-5-(2-hydroxy-3-(*tert*-butylamino)propoxy)-2,3-naphthalenediol; VII — 5,8-dihydronaphthol; VIII — acetonide of *cis*-5,6,7,8-tetrahydro-1,6,7-naphthalenetriol; IX — *cis*-5,6,7,8-tetrahydro-1,6,7-naphthalenetriol; X — 1,3-bis-(acetonide-*cis*-5,6,7,8-tetrahydro-1-oxy-6,7-naphthalenediol)-2-propanol.

pair of diastereomers. The sample of X from Farmos (Turku, Finland) gave a mass spectrum consistent with its structure. It contained an impurity which gave a response on the HPLC system about equal to that of X. Raw materials were obtained directly from manufacturers and tablets were purchased from a local pharmacy. The samples were coded by letters to represent manufacturers followed by a number to distinguish different lots from the same manufacturer.

Apparatus

The HPLC system (Varian Vista 5560) was fitted with a variable wavelength detector set at 219 nm (Varian UV 200), an autosampler (Varian 8085) and data processor (Varian Vista 402). Spherisorb 3-μm nitrile bonded phase columns (150 × 4.6 mm, Chromatography Sciences, Montreal) were used at ambient temperature with a flow rate of about 1.5 ml min^{-1}. Other equipment used was a UV–vis spectrophotometer (Varian DMS 90)

connected to a computer (Hewlett–Packard HP-85) and a plotter (Hewlett–Packard HP7470A), and an autotitrator (Mettler DL40RC Memotitrator) equipped with a 5 ml burette (DV 405) and a glass calomel electrode (DG 112).

Method for Related Compounds and Assay

Mobile phase

Sodium acetate (pH 3.4; 0.1 M)–acetonitrile (90:10, v/v). The column was conditioned with mobile phase for 24 h before use.

Solutions

The drug raw material was dried under vacuum at 60°C for 3 h. The following solutions were prepared in methanol: resolution solution (0.05 mg ml^{-1} racemate B (V), accurately known, 0.05 mg ml^{-1} III and 0.015 mg ml^{-1} VII); standard solution (4 mg ml^{-1} nadolol, accurately known); test solution (4 mg ml^{-1} nadolol, accurately known). The standard and raw material test solutions were prepared by transferring an appropriate amount of nadolol to a volumetric flask, filling to about 90% with methanol, sonicating for 5 min, cooling and diluting to volume with methanol. The tablet test solution was prepared as follows: 20 nadolol tablets were weighed, powdered, and an accurately weighed portion of the powder equivalent to 200 mg of nadolol was transferred to a 75-ml centrifuge tube, 50.0 ml of methanol was added, the centrifuge tube was capped, shaken horizontally for 15 min, centrifuged and filtered (Genex Autovial Syringeless Filters for aqueous and organic substances in the pH range 3–10 are suitable).

System suitability

Six 10-μl aliquots of the resolution solution were injected into the chromatograph. The system was deemed to be suitable for use if the resolution between III and racemate B was not less than 2.0, the resolution between III and VII was not less than 1.0, the relative standard deviation of peak areas due to VII was not more than 5% and the efficiency of the column, calculated from the peak due to VII, was not less than 6000 plates m^{-1}. The retention times of III, VII and racemate B were about 6, 4 and 8 min, respectively. For assay, the system was considered to be acceptable if the relative standard deviation of the peak area response of six injections of the standard solution was 2.0% or less.

Procedure

Impurities. Individual 10-μl aliquots of the test and resolution solutions, and a suitable blank, were injected into the chromatograph and the chromatogram recorded for 35 min. The percentage of each impurity in the test solution was calculated from $[100(A_i/A_r)(C_r/C_u)]$, where A_i is the area of the peak due to the individual impurity, A_r is the area of the peak due to racemate B in the resolution solution, C_r is the concentration of racemate B in the resolution and C_u is the concentration of nadolol in the test solution. Impurities that eluted too close to the solvent front to be integrated accurately were quantitated by $[100(H_i/H_r)(C_r/C_u)]$, where H_i is the peak height due to the individual impurity and H_r is the height of the peak due to racemate B in the resolution solution.

Assay. Equal quantities (10 μl) of the standard and test solutions were injected into the chromatograph and the chromatograms run for 35 min. The percentage of nadolol was calculated from $[100(A_u/A_s)(C_s/C_u)]$, where A_u and A_s are the areas of the nadolol peak in the test and standard solutions, respectively, and C_s and C_u are the concentrations of nadolol in the standard and test solutions, respectively.

Method for Racemates A and B

Mobile phase

A 40 ml volume of acetonitrile was transferred to a 1-l volumetric flask which was made up to volume with sodium acetate (pH 3.4; 0.1 M) and passed through a 0.45 μm filter. The column was conditioned for 24 h.

Solutions

The following solutions were made with methanol–racemate standard solution (0.1 mg ml^{-1} nadolol standard in which racemate A is accurately known); racemate test solution (0.1 mg ml^{-1} nadolol, accurately known); racemate tablet test solution (0.1 mg ml^{-1} nadolol prepared by dilution of the tablet test solution, prepared as described above).

System suitability

Six 10-μl aliquots of the standard solution were injected into the chromatograph. The

system was considered ready for use if the resolution between racemates A and B was not less than 0.6; the efficiency of the column, calculated using the peak due to racemate A was not less than 6000 plates m^{-1} and the relative standard deviation of the peak responses was not more than 3%. The retention times of A and B are typically 15 and 16.5 min, respectively.

Procedure

Separate 10-μl aliquots of the test and standard solutions, and a suitable blank, were injected into the chromatograph and the chromatogram recorded for 25 min. The percentage of racemate A in the test solution was calculated from $[100\ (A_a/A_w)(C_w/C_n)]$, where A_a is the peak area due to racemate A in the test solution, A_w is the peak area due to racemate A in the standard solution, C_w is the concentration of racemate A in the standard solution and C_n is the concentration of nadolol in the test solution.

UV spectra

The UV spectra of nadolol and related compounds were measured in methanol.

Discussion

Chromatography

A chromatogram obtained by the method for related compounds and assay is presented in Fig. 2. All available related compounds were resolved from each other, and the drug. Only one peak was observed for nadolol at the test solution concentration for this method but partial resolution of the nadolol racemates occured at concentrations of 0.1 mg ml^{-1} nadolol which made it impractical to use the drug for the quantitation of impurities. A change in mobile phase composition (method for racemates A and B) increased the resolution of the racemates (Fig. 3), with B eluting ahead of A. All available related compounds were resolved from the racemates.

UV and HPLC characteristics

The UV absorbance of nadolol and the available related compounds at 219 nm is given in Table 1. Based on duplicate weighings with multiple dilutions, the response of the HPLC system to nadolol and the related compounds was linear from the minimum quantifiable amount to 5% (the minimum quantifiable

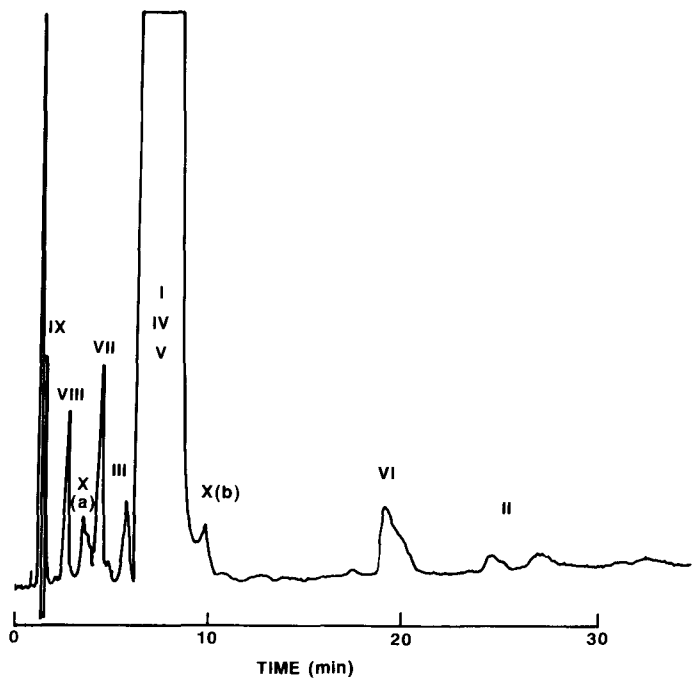

Figure 2
Chromatogram of nadolol and related compounds. The amounts on column were about 40 μg nadolol and 0.1 μg of each of the related compounds.

Table 1
UV and HPLC characteristics of nadolol-related compounds

Compound	UV characteristics			HPLC characteristics		
	Conc* ($\mu g\ ml^{-1}$)	Absorbance (219 nm)	Relative absorbance	RRT†	Relative‡ response	Minimum§ quantifiable (%)
Nadolol‖	20.0	0.601	1.00	1.00	1.00	0.05
II	19.0	0.519	0.91	3.03, 3.32	0.84	0.25
III	19.1	0.612	1.07	0.71	1.02	0.03
V	19.9	0.719	1.20	1.00	0.98	0.05
VI	19.0	0.729	1.27	2.41, 2.50	0.92	0.30
VII	20.2	1.154	1.90	0.54	1.91	0.03
VIII	19.8	0.813	1.37	0.34	1.06	0.01
IX	21.4	0.887	1.38	0.19	2.47	0.05
X	20.5	0.763	1.24	0.45, 1.35	0.76	0.25

*Solutions were made up in methanol.
†Retention time relative to the mean of the nadolol racemates at 8.1 min.
‡Area response relative to nadolol, except IX is relative to peak height.
§Minimum quantifiable amount, as a percentage of the amount of nadolol injected.
‖Nadolol, II and VI were mixtures of racemates or diastereomers which were partially resolved and X contained about 50% of an impurity. Relative responses are total responses for both peaks.

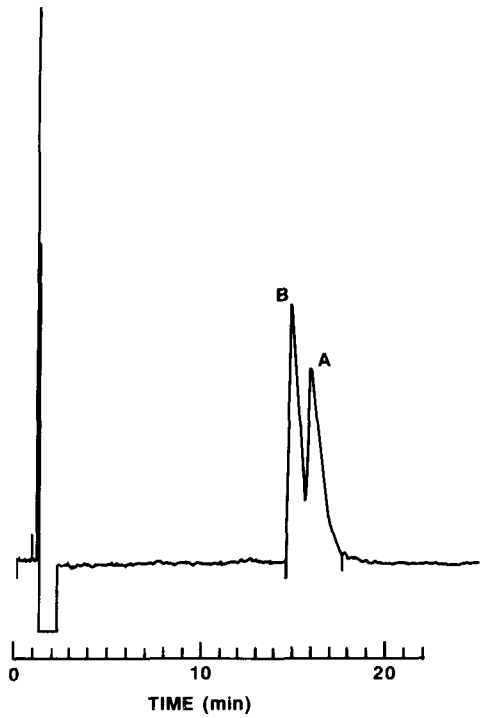

Figure 3
Chromatogram showing the resolution of the A and B racemates of nadolol. The amounts on column were about 0.5 μg of each.

amount to 0.5% for IX) of nadolol in the raw materials test solution. It was also linear from 50 to 150% of the concentration of nadolol called for in the assay procedure. Relative retention times, relative response and minimum quantifiable amounts of the nadolol isomers and the available related compounds are given in Table 1. IX elutes too close to the solvent front for accurate peak area integration, but can be quantitated by peak height comparison to V in the system suitability solution.

The linearity of response to the nadolol racemates, singly and in the presence of each other, was linear over a wide concentration range (Table 2). The apparently low response to racemate B occurred because the available sample contained about 15% of racemate A. Allowing for the racemate A content, the slopes of the two racemates are identical.

Precision

The precision of the system was determined by making six replicate injections of the assay standard solution at various times. The relative standard deviation of the nadolol peak responses ranged from 0.12 to 1.4%. The precision of the method was determined by six assays of sample A1. The mean was 101.0% with a relative standard deviation of 0.46%.

Stability of solutions

The relative peak areas of nadolol and the

Table 2
Linearity of response to racemates A and B

Compound	Range (%)	Slope ($\times 10^5$) (cts per μg injected)	$(rI)^2$
Racemate A	20–100	2.54	0.955
Racemate B	20–100	2.19	0.999
A in nadolol	12–84	2.48	0.991
B in nadolol	16–88	2.26	0.999

available related compounds in methanol at room temperature, injected at intervals over a period of 24 h, did not change. No increase in impurities was observed in solutions of nadolol stored in stoppered, clear glass volumetric flasks at room temperature over a period of 6 weeks.

System suitability parameters

System suitability requirements are a feature of many chromatographic methods. Their purpose is to ensure that the chromatographic system is sufficiently discriminating, and adequate in all respects for the test to be done. System suitability requirements are typically cast in terms of peak resolution, peak characteristics and precision. They should be minimum values to avoid unnecessary cost and delay.

Parameters for system suitability were monitored during development and use of the method for related compounds (Table 3) described in this report and are the basis of the system suitability requirements stated in the experimental section. Similar data served as the basis of the system suitability requirements for the method for racemates.

Ruggedness

A decrease in the amount of acetonitrile in the mobile phase resulted in an increase in the retention time of nadolol and all related compounds, while an increase above 20% leads to a loss of resolution between compounds VII, III and nadolol. When methanol was introduced into the mobile phase, retention times decreased. None of these changes had much effect on peak shape. The separations are pH-dependent. At pH 3.2, compounds III and VII coelute while at pH 3.6 III is unresolved from the drug.

A second column from the same manufacturer gave results similar to those obtained during method development.

Three tablet product samples were extracted for periods of 15, 30 and 45 min prior to analysis by the HPLC method. In no case were there significant differences in the amount of nadolol recovered. The method calls for a 15 min extraction.

Table 3
System suitability test results (related substances and assay)

	Retention time (min)			Resolution*			Efficiency		RSD†	RSD
Date	III	V	VII	III/V	III/VII	VII/V	V	VII	V	VII
Column 118813										
11/90‡	4.86	5.81	3.85	2.4	2.6	5.5	28083	12870	0.97	0.96
11/90	5.68	10.20	4.26	7.8	2.9	12.1	32109	12391	1.98	0.77
11/90	4.59	9.06	3.71	11.1	2.6	13.0	39281	11864	2.38	2.15
11/90	5.57	8.47	4.23	5.7	2.8	10.1	36392	12869	0.80	0.58
11/90	5.87	8.99	4.34	5.7	3.1	10.2	32595	12853	5.34	2.30
11/90	5.14	7.17	4.09	4.5	2.5	8.1	32592	12711	2.29	4.93
11/90	6.23	8.80	4.49	4.5	3.4	9.2	30295	12391	1.45	1.86
03/91	5.84	9.41	4.43	6.2	2.2	9.5	44147	6093	3.01	4.71
03/91	5.51	7.83	4.17	4.8	2.8	9.1	39533	11793	0.49	1.61
07/91	6.01	8.81	4.70	5.4	2.6	9.9	44493	14733	1.02	1.31
08/91	4.77	6.65	3.56	4.5	2.8	9.1	45007	10547	0.69	0.90
09/91	4.82	7.70	3.61	6.4	2.7	10.4	41393	8727	1.45	4.06
09/91	4.54	6.84	3.48	5.5	2.6	9.2	35647	10007	0.63	1.32
09/91	4.82	7.61	3.61	5.8	2.8	9.6	28987	9947	1.31	0.74
Column 039056										
09/90‡	5.53		4.94		1.0	5.5		7259		
Column 039054										
10/90	5.62	7.42	4.80	3.6	1.1	5.4	33516	10600		
09/91	4.52	7.40	3.81	6.2	1.5	7.9	29427	7013	1.17	1.46
Column 088902										
09/91	5.62	7.80	4.18	4.6	3.1	9.6	42913	13940	2.29	0.79

*Resolution and efficiency (plates m^{-1}) were calculated according to BP procedures.
†This is the relative standard deviation of the peak responses.
‡Related compound III was not resolved from the drug.

Table 4
Related compounds in nadolol raw materials (%)

RRT*	A1	A2	A3	A4	A5	B1	B2	C
0.22–0.28	0.09†	0.09	0.05	0.53	0.50		0.07	0.11
0.40							0.13	
0.46					0.36			0.23
0.52–0.55		0.07‡	0.08	0.43				
0.66								0.02
0.77	0.02	tr	0.02					
0.78						0.06		
2.6								0.05
2.8								0.38
4.2§			tr				0.22	
4.6							0.06	tr
4.7			0.06					
Total	0.11	0.16	0.21	0.96	0.86	0.06	0.48	0.79

*Retention times relative to nadolol at about 7 min.
†This relative retention time corresponds to that of IX.
‡The relative retention time corresponds to that of Xa.
§The relative retention times of IIa and IIb are about 4.2 and 4.6, respectively.

Results

Impurities in raw materials

Eight samples from three manufacturers were analysed. Total impurities, given in Table 4, ranged between 0.1 and 1.0%; the largest single impurity, which had a retention time similar to that of IX, was present at a level of about 0.5%.

Assay of raw materials and tablets

Eight raw material and three tablet samples were analysed in triplicate for total nadolol content. For raw materials, mean results in per cent, with relative standard deviations in parentheses, were: A1, 101.0 (0.46); A2, 100.4 (0.21); A3, 100.2 (0.43); A4, 99.1 (0.41); A5, 100.2 (0.84); B1, 99.5 (0.70); B2, 97.7 (0.70); and C, 98.7 (0.89). For tablet samples the results, as a percentage of label claim, were: CC1, 96.2 (0.37); DD1, 98.4 (0.16); and EE1, 96.9 (0.20).

Assay for racemate content

Partial resolution of the nadolol racemates by the assay method prompted development of an HPLC method for quantitation of the racemates in raw materials and tablets. The results in Table 5 indicate that several samples may not be 1:1 mixtures of the two racemates, and may fall outside the USP acceptable range of 40–60% racemate A, when determined by infrared spectroscopy. USP attributes absorbance at 7.90 μm to racemate A and absorbance at 8.05 μm to racemate B. The infrared spectrum exhibits peaks at 7.90, 8.00 and 8.10 μm, but absorption at 8.05 μm is a shoulder in some cases and a peak in others. Results concordant with those obtained by HPLC were obtained when the peaks at 7.90 and 8.00 μm were taken as representative of the racemates (Table 5).

Table 5
HPLC and infrared determination of racemate A (%)

Sample	HPLC	Infrared*
A1	51; 52	47
A2†	49	41; 32; 40
A3	43; 46	36; 40; 39
A4	52	
A5	53	
B1	31; 32; 32; 32	29; 30
B2		48; 48
C‡	52	50; 50
F-1§		50
CC1	54; 53; 51	
DD1	52; 52; 52	
EE1	53; 54; 50	

*Calculations based on absorbances at 7.90 and 8.00 μm.
†Samples A2 and A3 were visibly granular.
‡Racemate A content determined in duplicate from absorbances at 7.90 and 8.05 μm was 64 and 68%. It was also 64% for sample F-1.
§This is USP Reference Standard Nadolol, Lot F-1.

Conclusions

The methods described in this report can be used to determine total nadolol, related com-

pounds and racemate composition of drug raw material. The racemate composition of drug in tablets can also be determined, an important feature for post-marketing surveillance.

Nadolol cannot be used as the standard to quantitate impurities as partial resolution into its racemates results in inconsistent peak integration. The results in this report were obtained using racemate B as the standard (A could equally well have been used), and III for system suitability. If the racemates and III were not available, nadolol and VII can be used for resolution and VII for quantitation. However, the system response to VII is about twice that due to equal concentrations of the other available related compounds, and an appropriate adjustment should be made (Table 1).

Compound IX elutes close to the solvent front and is difficult to quantitate by peak area, but its response can be measured by peak height. Its relative response is about twice that of racemate B.

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