DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 17(13), 1843-1855 (1991)

VALIDATION OF METHODS FOR THE ASSAY OF FLURBIPROFEN AND FLURBIPROFEN SODIUM, RELATED COMPOUNDS AND VOLATILE IMPURITIES IN RAW MATERIALS AND TABLETS

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ABSTRACT

A high performance liquid chromatographic method has been developed for the assay of flurbiprofen, or flurbiprofen sodium and related compounds in drug raw materials and tablets. A phenyl column with a mobile phase of acetonitrile: 1.0% acetic acid (60:40) provide for the resolution of twenty-one related compounds from the drug. Minimum detectable levels of the related compounds are 0.01% and minimum quantifiable levels are 0.1% or less. Total impurity levels in seven raw materials ranged from 0.0 to 0.6%. One impurity, 2-(4-biphenylyl) propionic acid, is present in most samples at about 0.3%. A gas chromatographic method was developed for organic volatile impurities

INTRODUCTION

Drug raw materials are frequently produced in different countries around the world by different synthetic routes. It is essential to have validated methods for purity and assay which respond to the presence of impurities originating from known synthetic and degradation pathways. The USP[1] contains monographs for flurbiprofen sodium raw material and ophthalmic solution and the BP[2] contains monographs for flurbiprofen raw material and tablets. The USP limits 2-(4-biphenylyl) propionic acid to a maximum of 1.5% in flurbiprofen sodium raw materials by an HPLC method, but has no other limits on related compounds. The BP requirements by HPLC, for related compounds in flurbiprofen raw material are 0.5% for 2-(4-biphenylyl) propionic acid and any other individual impurity and not more than 1.0% for the total of all impurities.

The structures of flurbiprofen and the related compounds, used to evaluate the USP and BP methods and to validate the modification described in this report, are presented in Figure 1. The USP method for 2-(4-biphenyl) propionic acid, based on a C-8 column, does not resolve VIII from the drug and absorbance at the specified wavelength of 280 nm was too weak for quantitation of most of the available related compounds. The BP method, based on a C-18 column with detection at 254 nm, gave extremely long retention times for several of the available related compounds. High noise levels, attributed to the presence of 5% acetic acid in the mobile phase, made it impossible to integrate several of the related compounds at the 0.1% level.

The method described in this report overcomes these difficulties. All available related compounds were

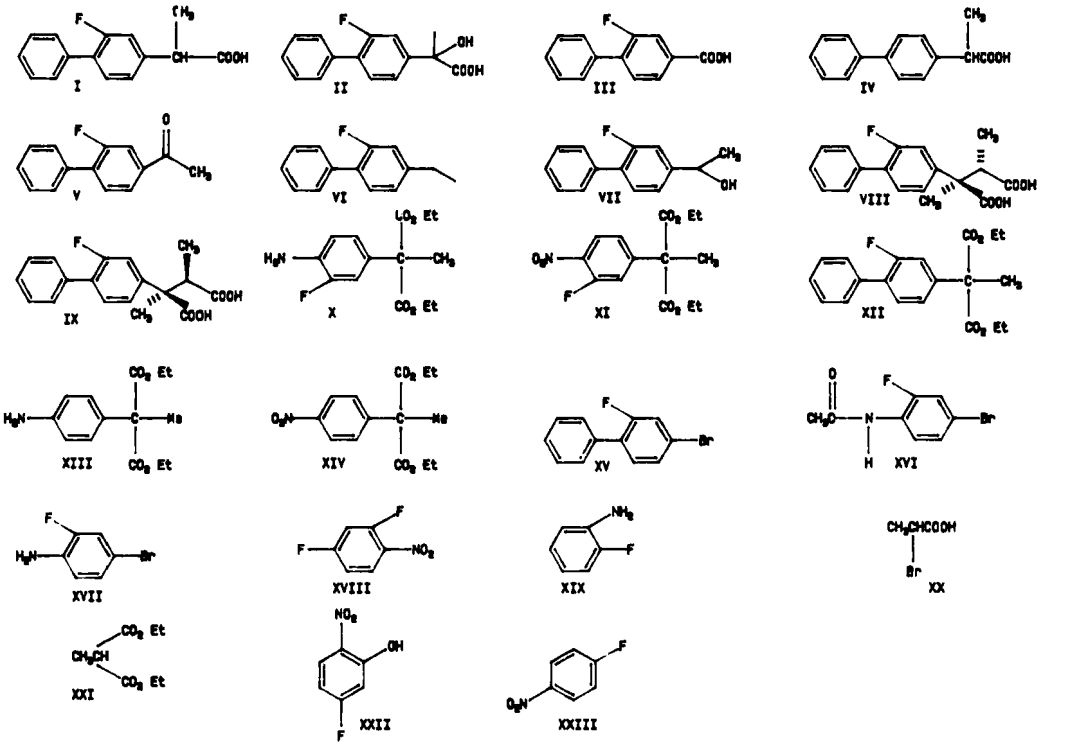

FIGURE 1
Structures of flurbiprofen and related compounds: I, 2-(2-fluorobiphenyl-4-yl)propionic acid (flurbiprofen); II, 2-(2-fluoro-4-biphenylyl)-2-hydroxy propionic acid; III, 2-fluorobiphenyl-4-carboxylic acid; IV, 2-(4-biphenylyl)propionic acid; V, 4-acetyl-2-fluorobiphenyl; VI, 4-ethyl-2-fluorobiphenyl; VII, 2-fluoro-4-(1-hydroxyethyl)biphenyl; VIII, cis-2-(2-fluoro-4-biphenylyl)-2,3-dimethyl succinic acid; IX, trans-2-(2-fluoro-4-biphenylyl)-2,3-dimethyl succinic acid; X, diethyl-(3-fluoro-4-aminophenyl)-methyl malonate; XI, diethyl-(3-fluoro-4-nitrophenyl)-methyl malonate; XII, diethyl-(2-fluorobiphenyl-4-yl)-methyl malonate; XIII, diethyl-2-(4-aminophenyl) methyl malonate; XIV, diethyl-2-(4-nitrophenyl) methyl malonate; XV, 4-bromo-2-fluoro-biphenyl; XVI, 4-bromo-2-fluoro-acetanilide; XVII, 4-bromo-2-fluoroaniline; XVIII, 2,4-difluoronitrobenzene; XIX, 2-fluoroaniline;XX, 2-bromopropionic acid; XXI, diethyl methyl malonate; XXII, 5-fluoro-2-nitrophenol; XXIII, 1-fluoro-4-nitrobenzene

completely separated from the drug and partially or completely separated from each other. Two compounds, XX and XXI, could not be detected due to the lack of a UV chromophore.

MATERIALS AND METHODS

All compounds were used as received. Flurbiprofen related compounds were obtained as follows: I to X and XV to XVII (HPB chemical laboratory); IV (BP); I,X, XI and XII (Nobel Chemicals S.R.L., Milan, Italy); I and X to XIV (Erregierre, San Paolo d'Aragon, Italy); XVIII to XXIII and potassium hydrogen phthalate ACS (Aldrich Chemical Company, Milwaukee, Wisconsin). Acetonitrile, HPLC grade (J.T. Baker Co., Phillipsburg, N.J.), glacial acetic acid (Fisher Scientific Company, Fair Lawn, N.J.) and 0.1 N sodium hydroxide (Anachemia Chemicals Inc, Champlain, N.Y.) were used. Water was deionised using a Sybron/Barnstead system.

The HPLC system (Waters pump Model 510) was fitted with a 5-µL loop injector (Rheodyne No 7120), a progr-ammable UV detector (Waters 490) set at 254 nm and an integrator (Varian 4270). A phenyl bonded phase column (Waters Novo-Pak, 5-µm, 150 X 3.9 mm, No T82871) was used at ambient temperature with a mobile phase flow rate of 1.0 mL/min.

A Hewlett Packard 5890 gas chromatograph equipped with a model 7673A autosampler and a model 3393A integrator with a HP 9114B disk drive was used for initial detection and quantitation of volatile impurities. It was fitted with a deactivated fused silica retention gap (1 m X 0.53 mm) coupled to a fused silica capillary column (5% phenyl dimethylpolysiloxane,

1.5 µm, 30 m X 0.53 mm, J&W Scientific). Infrared spectra were obtained using a Digilab FTS-40 FTIR coupled to a Hewlett Packard 5890 gas chromatograph equipped with a dimethylpolysiloxane column (2.65 µm, 7 m X 0.53 mm). Other equipment used was as follows: UV-VIS spectrophotometer - Varian DMS 90 connected to a HP 85 computer with a plotter and disk drive; pH meter - Fisher Accumet Model 425; balance - Mettler.

LC Methods

Mobile Phase: Transfer 400 mL acetonitrile and 10 mL glacial acetic acid to a 1 L volumetric flask, dilute to volume with deionized water and mix. Filter through a 0.45 µm filter and degas under vacuum.

Dissolution solution: Transfer 400 mL acetonitrile to a 1-L volumetric flask, dilute to volume with water and mix. Use dissolution solution for all solutions.

Related compounds standard solution - 0.004 mg/mL flurbiprofen and 0.01 mg/mL 2-(4-biphenylyl) propionic acid.

Related Compounds test solution - 2.0 mg/mL flurbiprofen or flurbiprofen sodium dihydrate.

Assay standard solution - 0.1 mg/mL (accurately known) flurbiprofen standard.

Assay test solution - 0.1 mg/mL (accurately known) flurbiprofen or flurbiprofen sodium.

Tablet related compounds test solution - Powder 20 tablets into a uniform composite. Accurately weigh an amount equivalent to about 50 mg of flurbiprofen into a 50-mL test tube fitted with a teflon-lined screw cap. Add 25.0 mL of dissolution solution, shake for 30 minutes and centrifuge at 2000 rpm for 10 min.

Tablet assay test Solution: Dilute an aliquot of the

Tablet related compounds test solution to a final concentration of about 0.1 mg/mL.

System Suitability: Inject a 5-µL aliquot of the Related compounds standard solution; the resolution is greater than 2.0, the efficiency of the column (drug peak) is more than 45 000 plates/meter and the tailing factor is less than 1.5. Six replicate injections of the Related compounds standard solution give a coefficient of variation of less than 5.0%. The retention time of flurbiprofen is between 8 and 11 minutes and the RRT of IV is about 0.9. Adjust the water content of the mobile phase to meet these requirements. Five replicate injections of the assay standard solution give a coefficient of variation of less than 1.0%.

PROCEDURE

Related compounds in flurbiprofen - Inject separately 5 µL of the Related compounds standard and test solutions into the chromatograph and allow a run time of 90 minutes. Calculate the percentage of each impurity in the Test solution from

$$100(A_i/A_s)(C_s/C_u)$$

where A_i is the peak response due to each individual impurity in the Test solution, A_s is the peak response due to flurbiprofen in the Standard solution and C_u and C_s are the concentrations of flurbiprofen in the Test and Standard solutions, respectively. Calculate the quantity of 2-(4-biphenylyl) propionic acid in the Test solution from

$$100(A_b/A_f)(C_f/C_u)$$

where A_b and A_f are the areas of the peaks due to 2-(4-biphenylyl) propionic acid in the Test solution and Standard solutions, respectively, and C_f is the

concentration of 2(4-biphenylyl) propionic acid in the Standard solution.

Drug Assay

Note: Dry the standard and samples at 60°C over phosphorus pentoxide for 4 hrs under vacuum.

Inject 5-µL aliquots of the Standard solution and the Assay or Tablet Assay test solution into the chromatograph and run for 15 minutes. Calculate the percentage of flurbiprofen from

$$100(A_u/A_s)(C_s/C_u)$$

where A_u and A_s are the areas of the flurbiprofen peaks due to the Test and Standard solutions, respectively and C_s and C_u are the concentrations of flurbiprofen in the Standard and Test solutions, respectively. If the Test solution contains flurbiprofen dihydrate, the above equations are multiplied by (302.28/244.26) the molecular weight ratio of the sodium dihydrate salt over the drug.

GC method

Sample solution - Accurately weigh about 17 mg of flurbiprofen into a 1.7 mL vial, crimp the vial top and add 1.7 mL of benzyl alcohol.

Procedure

Set the carrier gas flow rate to 6.5 mL/min, the nitrogen makeup gas to 27 mL/min, the air flow to 275 mL/min and the hydrogen flow to 34 mL/min. Inject 1.0 µL each of blank (benzyl alcohol) and Sample solution in the splitless mode with the purge at about 77 mL/min, activated at 0.8 min. Temperature-program the column oven thus: 35°C for 4 min; 5°C/min to 50°; 20°C/min to 150°C and hold for 15 min. If peaks due to organic volatile compounds are present, make a tentative identification by comparison to a table of retention times of common organic volatile impurities. If necessary, confirm the identification by the procedure for Volatile impurity identification given below.

Quantitate by peak area comparison to an external standard of the compound.

Identification of Volatile Impurities

Transfer 0.5 g flurbiprofen and 50 µL water to a 4 mL vial and heat at 100°C for 15 min. Inject approximately 3 mL of headspace gas from the vial into the gas chromatograph coupled to a FTIR detector. Identify the unknown impurity by comparison of its spectrum to similar spectra in the FTIR library.

RESULTS AND DISCUSSION

The HPLC method described in the USP monograph for flurbiprofen sodium is used to support a limit for IV rather than a limit for all related compounds. The method was evaluated to determine if it would separate twenty-one flurbiprofen related compounds from the drug. The column called for, a Spherisorb C8, 300 x 4.0 mm, was not available so a similar column, Brownlee Spheri-10 RP-8, 250 x 4.6 mm, No 10624, was used. It met the system suitability test requirements. Under these conditions, VIII was unresolved from the drug. In addition, the specified wavelength of 280 nm is removed from the absorbance maximum of most related compounds (Table 1), so that several could not be quantitated at the 0.2% level.

The BP HPLC method for related substances was examined using a CSC-S ODS2, 3-µm, 150 x 4.6 mm column (No 118859), which was the closest available to the one specified, a Waters Resolve C18, 5 µm, 150 x 4.6 mm column. The BP mobile phase is 35 volumes of acetonitrile and 5 volumes of glacial acetic acid in 60 volumes of deionized water. Due to the high concentration of glacial acetic acid, and subsequent low pH of about 2.2, noise levels in the detector were high.

Table 1

Linearity Data for Flurbiprofen and Related Compounds and UV Maxima.

Compound	Maxima nm	RRT[1]	Slope[2]	Relative Response	Intercept[3]	Quant'n Limit(%)
I	246	1.0	11377	1	-2738	.025
II	247	0.45	12081	1.06	-16006	.03
III	263	0.67	18631	1.64	-7606	.03
IV	253	0.86	15447	2.08	-8362	.025
V	276	1.33	11061	0.97	-848	.025
VI	245	4.75	14142	1.24	-39592	.05
VII	245	0.74	16537	1.45	-2941	.025
VIII	246	0.62	13884	1.22	-3214	.025
IX	247	0.56	9224	0.81	-3467	.025
X	240	0.77	2470	0.22	-1465	.025
XI	260	2.15	2510	0.22	-215	.03
XII	247	6.47	8958	0.79	-69360	.1
XIII	244	1.47	1479	0.13	-2510	.05
XIV	269	1.76	3233	0.28	-3893	.05
XV	248	3.96	13401	1.18	-13332	.05
XVI	243	0.29	7980	0.70	-1332	.025
XVII	237	0.49	4349	0.38	-1350	.025
XVIII	258	0.49	6720	0.59	-1814	.025
XIX	228	0.30	801	0.07	-464	.03
XX	200					
XXI	209					
XXII	277	0.42	2811	0.25	-915	.025
XXIII	267	0.47	6461	0.57	-9119	.025

[1] Retention time relative to flurbiprofen at 9.9 min.
[2] Area counts per ng.
[3] Area counts.

This caused a decrease in sensitivity to the point where some impurities at the 0.1% level could not be integrated. In addition, retention times were long, in excess of 120 minutes for XII. When the specified column (Resolve C18, 150 X 3.9 mm, 5 µm, Waters serial No T83471) did become available, the results obtained were similar to those described above. Due to these problems, a modified method was developed and validated.

A representative chromatogram showing the resolution of the related compounds from flurbiprofen is given in

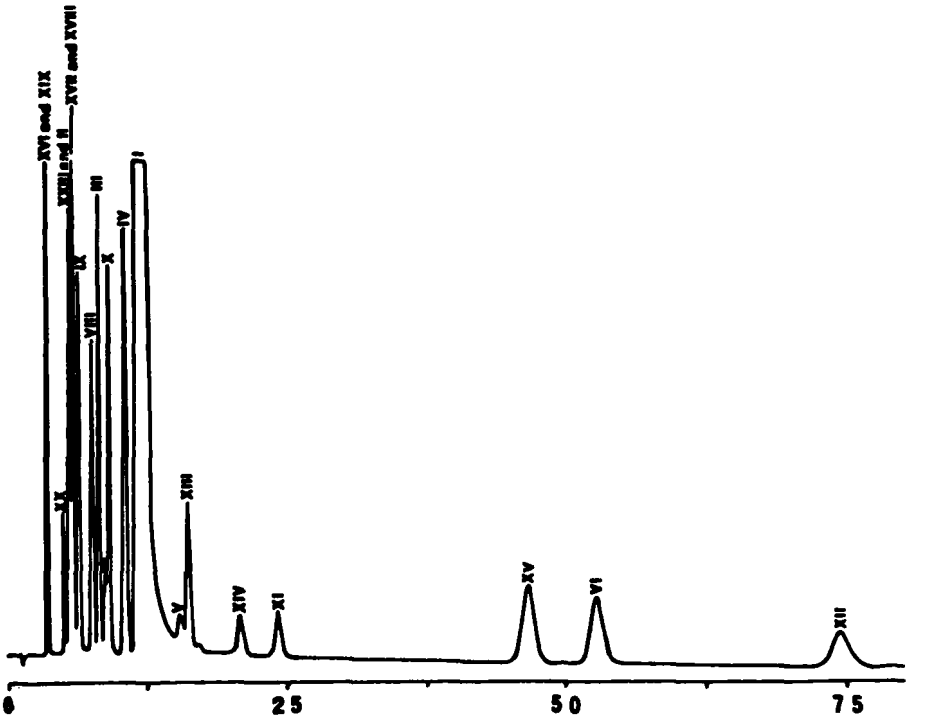

Figure 2

Chromatogram of a mixture of flurbiprofen (10 µg on column) and available related compounds at levels of 0.18 to 0.20%, relative to the drug. The time scale is in minutes.

Figure 2. The precision of the system was determined by making six replicate injections of a solution containing 0.01 mg/mL flurbiprofen. The coefficient of variation was 3.1%. A solution of flurbiprofen, 0.091 mg/mL in 40% acetonitrile in water, gave a coefficient of variation of 0.4%. The system response to the related compounds was linear up to about 1% (R^2 =.99), based on the concentration of drug called for by the method (Table 1). The response to the drug in the assay concentration range was linear from 0.01 to 0.28 mg/mL (R^2 = 0.999).

Table 2

Related Substances in Flurbiprofen Raw Material and Tablets (%)

RRT[1]	Aa	Ba1	Ba2	Ca	Ds1	Ds2	Ds3	AAa	BBa
.37	.01		.01						
.46						.08	.01		
.49			.01						
.63						.06	.02	.01	
.65				.02					
.80	.03								
.89[2]	.11		.03	.33	.34	.34	.37	.40	.44
1.6	.05								
6.1[3]	.13		.20						
Total:	.33	0	.25	.35	.48	.37	.38	.40	.44

[1] Relative retention time to flurbiprofen at 9.1 minutes.
[2] The RRT of IV is about 0.86.
[3] The RRT of XII is about 6.4.

Related compounds found in drug raw materials and tablets are given in Table 2. Samples were coded thus: each manufacturer was assigned a capital letter, an "a" or "s" to distinguish between flurbiprofen and flurbiprofen sodium respectively and a number to distinguished between samples from the same manufacturer, formulation were assigned two capital letter . The compound eluting at RRT 0.89 (possibly IV) was common to all raw materials except B. A second impurity, found in samples Aa and Ba2, may be XII, an immediate synthetic precursor to flurbiprofen. The precision of the HPLC method was checked by analyzing a tablet composite of BB six times on a single day to give a mean assay of 97.0% with a coefficient of variation of 0.5%. The same composite was analysed in triplicate on five different days. The mean value was 97.0%, with a coefficient of variation of 0.5%.

Table 3

Assay of Flurbiprofen and Flurbiprofen Sodium Raw Materials(%)

Assay Method	Aa	Ba1	Ba2	Ca	Ds1	Ds2	Ds3
Aqueous Titration							
	99.9	99.0	99.8	99.4			
	99.7	99.2	100.1	99.5			
Mean:	99.8	99.1	99.9	99.4			
HPLC	99.2	std	100.3	100.1	93.7	99.9	100.2
	99.6	std	100.1	99.9	99.7	98.6	99.2
	98.9	std	100.0	99.6	96.0	98.1	98.7
					98.7		
					98.5		
					97.3		
					97.0		
					96.9		
					95.1		
Mean :	99.2		99.9	100.1	97.0	98.9	99.4
C.V.	0.4		0.3	0.2	1.9	0.9	0.8

Table 4

Assay of Flurbiprofen Tablets by HPLC (%)[1].

Sample	Flurbiprofen Content						Mean (C.V.)
AA	90.7	91.8	90.6	92.1	93.6		91.8 (1.3)
BB day 1:	96.8	96.4	97.5	97.6	96.7	96.7	97.0 (0.5)
day 2:	95.6	96.7	96.9				96.4 (0.7)
day 3:	96.9	97.5	97.0				97.1 (0.3)
day 4:	97.7	98.0	98.4				98.0 (0.4)
day 5:	96.4	96.2	96.4				96.3 (0.1)
inter-day mean and c.v.							97.0 (0.7)

[1] Sample Ba was used as the standard

Assay results for flurbiprofen raw materials and tablets are given in Tables 3 and 4, respectively. The largest coefficients of variation observed were 1.9% (Ds1) for raw materials and 1.3% for tablets. HPLC assay results for flurbiprofen raw materials are compared with results from the BP titration procedure in Table 3.

Flurbiprofen drug raw material samples were analysed for volatile impurities by gas chromatography. No volatile compounds were observed in samples A and D. Based solely on a comparison of retention times, B may contain about 75 ppm acetone and C may contain benzene and trichloroethylene, but the levels were too low for confirmation by GC/FTIR. If both are actually present, they are well below the proposed USP limit of 100 ppm.

ACKNOWNLEDGEMENT

Thanks are expressed to N.M. Curran, R.W. Sears, K. Wilson and R.C. Lawrence for technical assistance, S.J. Graham for technical evaluation of the method and to J.C. Ethier, H. Beckstead and H. Avdovich for the mass spectra, infrared and NMR spectra, respectively.

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