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Note

Purity assay of niflumic acid by reversed-phase high-performance liquid chromatography

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Niflumic acid is a widely prescribed non-steroidal anti-inflammatory drug[1], for which the analytical methods available, including gas chromatography and high-performance liquid chromatography (HPLC), have been developed for bioavailability studies[2–6]. However, no method is available for the determination of the purity of niflumic acid. This paper describes an HPLC method for the determination of two impurities that appear during the synthesis of niflumic acid[7]: a precursor, 2-chloronicotinic acid (compound II), and a by-product, 2-hydroxy-N-[(3′-trifluoromethyl)-phenyl]nicotinamide (compound III) (Fig. 1). For the quality control of niflumic acid, HPLC proved superior to semi-quantitative assay by thin-layer chromatography (TLC) with respect to accuracy, reproducibility and sensitivity.

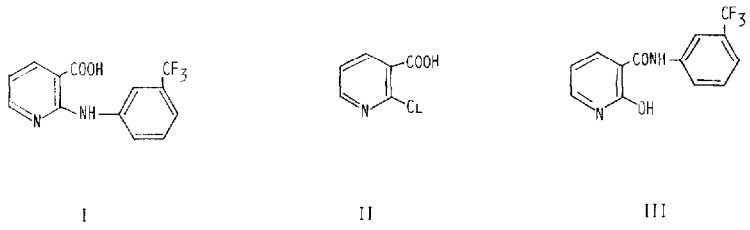

Fig. 1. Structural formulae of niflumic acid (I), 2-chloronicotinic acid (II) and 2-hydroxy-N-[(3′-trifluoromethyl)phenyl]nicotinamide (III).

EXPERIMENTAL

Equipment and materials

A Varian 5060 analytical pump coupled with a Varian UV100 UV detector and a Valco N60 injection valve (10 μl sample loop) was used. The chromatograms were plotted on a Hewlett-Packard 3390A integrator. The HPLC column was a pre-packed methyloctylsilyl (MOS) column (150 mm × 4.6 mm I.D.; particle size 5 μm) from Alltech. The solvents were of analytical-reagent grade and were filtered before use.

Chromatography

HPLC was carried out at a flow-rate of 1 ml/min with methanol–0.1% ortho-

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phosphoric acid solution (65:35) as the mobile phase. Wavelength programming was used to gain a higher sensitivity for each compound: nuflumic acid, II and III were detected at 289, 267 and 330 nm, respectively.

Preparation of standard solutions

A solution of 25 mg of II and 25 mg of III in 25 ml of methanol was prepared and diluted to obtain four standard solutions containing 10, 5, 2.5 and 1 μg/ml, respectively, of each compound. The volumes injected were 10 μl.

Preparation of sample solution

A solution of 50 mg of niflumic acid in 50 ml of methanol was prepared. The volume injected was 10 μl.

Calculation

Peak areas at 1.6 and 11.3 min were plotted against the amounts of II and III in the standard solutions. The peak areas for the sample solution were plotted on calibration graphs.

RESULTS AND DISCUSSION

Fig. 2 shows the chromatogram of a niflumic acid sample. The vertical lines indicate the wavelength setting. The sensitivity under the conditions described above for both impurities is, better than 5 ng, detected with a moderate signal attenuation.

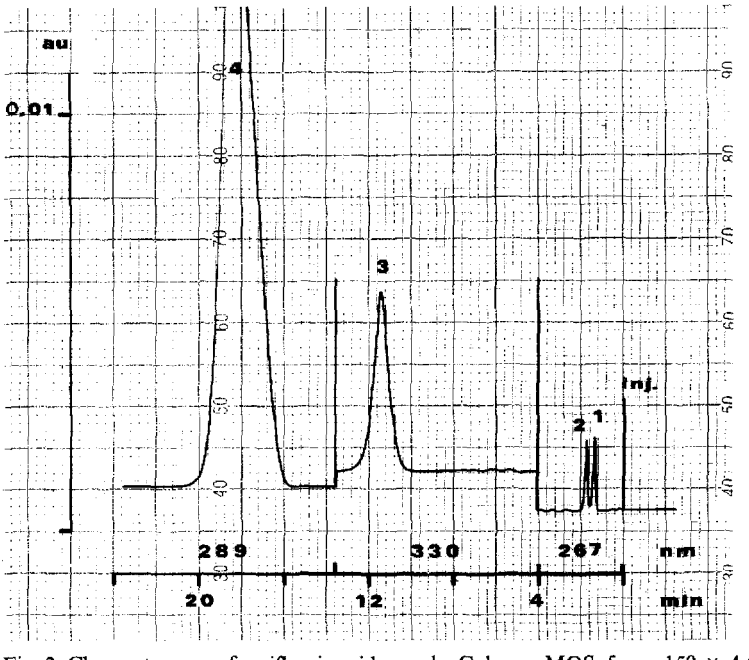

Fig. 2. Chromatogram of a niflumic acid sample. Column: MOS, 5 μm, 150 × 4.6 mm I.D. Mobile phase: methanol–0.1% orthophosphoric acid solution (65:35). Flow-rate: 1 ml/min. Peaks: 1 = solvent; 2 = II; 3 = III; 4 = I.

TABLE I

REPRODUCIBILITY OF THE DETERMINATION OF IMPURITIES IN NIFLUMIC ACID

Parameter	*Compound I*		*Compound III*	
Amount in niflumic acid (%)	0.2	0.1	0.5	0.2
Coefficient of variation (%)	2.3	3.9	1.4	2.4
No. of assays	4	4	3	3

Linearity, accuracy and reproducibility were studied. The excellent fits of the least-squares lines demonstrate the linearity of the relationship between concentrations of impurities and peak areas (for II $R > 0.999$; for III $R > 0.999$). The accuracy is illustrated by the magnitude of the standard deviations given in Table I. The results of the analysis of five commercial batches in two different laboratories, given in Table II, show the good reproducibility of the method.

TABLE II

RESULTS OF THE PURITY ASSAY ON FIVE COMMERCIAL BATCHES OF NIFLUMIC ACID IN TWO DIFFERENT LABORATORIES

Batch	*Compound II (%)*		*Compound III (%)*	
	Lab. 1	*Lab. 2**	*Lab. 1*	*Lab. 2**
1	0.02	0.02	0.24	0.26
2	0.07	0.06	0.51	0.49
3	0.04	0.05	0.95	0.96
4	0.02	0.02	0.49	0.50
5	0.02	0.02	0.70	0.72

* Experimental data supplied by the analytical control laboratory of UPSA Laboratories, Agen, France.

This accurate, specific and reproducible assay can be used in industrial process control and in pharmaceutical quality control and can satisfactorily replace the classical semiquantitative TLC purity assay. The method lends itself to automation for use in routine quality control laboratories, and has been included in a proposal to the French Pharmacopoeia Committee for a monograph on niflumic acid.

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REFERENCES

1 J. Goujeon, J. Moreau-Hottin, S. Bajolet and J. C. Etienne, *Therapie*, 23 (1968) 951.
2 T. Cowen and J. R. Salomon, *Method. Dev. Biochem.*, 5 (1976) 211.
3 A. Schumacher, H. E. Geissler and E. Mutschler, *J. Chromatogr.*, 162 (1979) 489.
4 H. Masaroni, M. Masako and T. Akio, *Yakuzaigaku*, 39 (1979) 87.
5 G. Houin, F. Bree and J. P. Tillement, *J. Chromatogr.*, 223 (1981) 351.
6 G. Houin, D. Tramblay, F. Bree, A. Dufour, P. Ledudal and J. P. Tillement, *Int. J. Clin. Pharmacol. Ther. Toxicol.*, 21 (1983) 130.
7 C. Hoffman and A. Faure, *Bull. Soc. Chim. Fr.*, 7 (1966) 2316.