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Chromatographic analysis of picotamide and its impurities

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Summary

Picotamide and its potential impurities in the raw material, which might derive from the synthetic process or be due to degradative pathways, were detected and quantified by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC) procedures. Good separations were achieved using a mobile phase of methanol and pH 7.9 phosphate buffer. Impurities from solvents were determined by a suitable gas chromatographic (GC) method.

Introduction

Picotamide, *N*,*N*-bis-(3-picolyl)-4-methoxyisophthalamide monohydrate (Fig. 1, **X**), is a new molecule (Samil, G-137) emerging out of the synthesis of numerous derivatives of 4-hydroxyisophthalic acid which are endowed with various pharmacological activities (Orzalesi et al., 1967, 1969; Selleri et al., 1968).

These studies have shown that some bis-amidic derivatives possess both an anti-clotting and fibrinolytic activity (Selleri et al., 1971) and a capacity for inhibiting animal (Orzalesi et al., 1975) and human (De Cunto et al., 1983) platelet aggregation in vivo. The most marked inhibitory action is found in picotamide, a compound which has shown itself in previous studies (Orzalesi et al., 1975) to lack any anti-inflammatory activity. The dissociation between platelet anti-aggregation and anti-inflammatory activities has led researchers to investigate the mechanism of action of this new compound.

Picotamide has been found not to act with a similar mechanism of action to that of acetylsalicylic acid, but to exert its effect by interfering with the prostaglandin endoplatelet mechanism through a blockade of tromboxane synthetases and possibly a blockade of tromboxane receptors (Berrettini et al., 1983). These studies have also demonstrated that 4-methoxyisophthalic acid, on the other hand, is able to activate arachidonic acid platelet aggregation (De Cunto et al., 1983).

In the light of this results it seemed interesting, while working out a suitable method for the detection and the determination of picotamide, to detect and quantitate potential impurities in the raw material which might derive from the synthesis procedure or be consequent on the degradation of the product itself.

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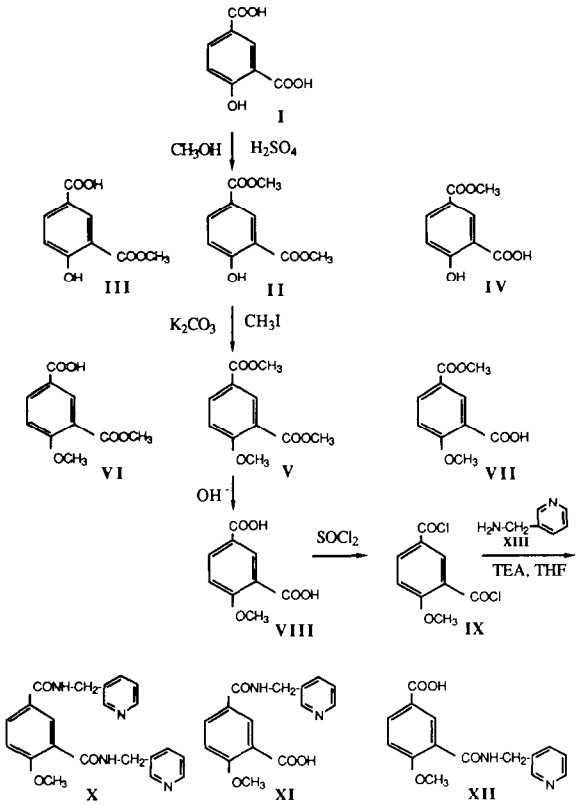

Fig. 1. Synthesis of picotamide (X) showing its potential impurities and by-products.

4-Methoxyisophthalic acid (**VIII**), the starting raw material for the synthesis, is in fact obtained by methylation of the phenol oxhydrile of dimethyl 4-hydroxyisophthalate (**II**) and subsequent hydrolysis; **VIII** is first chlorinated and then condensed with 3-picolylamine, according to the scheme in Fig. 1.

Thus the potential impurities deriving from the synthesis may be of 4 types:

(a) Compounds having free phenol oxhydrile: 3-carbomethoxy-4-hydroxybenzoic acid (**III**), methyl 3-carboxy-4-hydroxybenzoate (**IV**), and dimethyl 4-hydroxyisophthalate (**II**);
(b) Compounds of acid nature, having a methylated phenol oxhydrile: 3-carbomethoxy-4-methoxybenzoic acid (**VI**); 2-methoxy-5-carbomethoxybenzoic acid (**VII**); 4-methoxyisophthalic acid (**VIII**) and its relative monoamides in position 1 (**XI**) and 3 (**XII**). It should be noted that compounds **VI** and **VII**, which may be present after the first step of synthesis, are transformed by subsequent hydrolysis into compound **VIII** and do not therefore constitute potential impurities to be detected;
(c) Compounds of basic nature: 3-picolylamine (**XIII**); and
(d) Impurities of solvents: mainly methanol, ethanol, acetone.

Stability studies of the raw material, moreover, demonstrated that the impurities which may derive from degradation are compounds **VIII, XII** and **XIII** (Aylward et al., 1980).

Materials and Methods

Chemicals

Picotamide raw material and standard sample were obtained from SAMIL (Rome, Italy). The other compounds were prepared according to the method described previously (Hunt et al., 1956; Gladych and Taylor, 1956; De Cunto et al., 1983). All other solvents and reagents were of analytical grade.

TLC conditions

Merck F-254 silica gel plates 0.25 mm thick, were used; the mobile phase was made up of the following eluents (1) chloroform : methanol (9 : 1); (2) methanol : conc. ammonia (100 : 1.5); (3) benzene : ethylacetate : acetic acid (2 : 1 : 0.1) and (4) ethylacetate : ethanol (2 : 1).

The location was performed using UV light (254 nm), or a solution of ferric chloride, according to the compound to be detected.

HPLC conditions

A Perkin-Elmer Series 4 liquid chromatograph, equipped with an LC 85-B UV detector (254 nm), LC Autocontrol LCI 100 calculator/integrator and a Spherisorb S5 ODS1 column (25 cm × 4 cm, 6 mm i.d.), was used.

The mobile phase was a mixture of methanol and phosphate buffer pH = 7.9 (60 : 40). The flow rate was 0.8 ml · min^{-1}, chart speed 4 mm · min^{-1} and attenuation A × 32.

GC conditions

A Perkin-Elmer Model 990 gas chromatograph, equipped with a flame ionisation detector was used. The column (2 m × 2.2 mm i.d.) was packed with Carbowax 400 (15%) on Chromosorb (80–100 mesh); the instrument was operated with injector, detector and oven temperatures at 150 °C, 150 °C and 50 °C, respectively (3 min^{-1} program). Nitrogen was used as carrier gas at a flow-rate of 10 $ml \cdot min^{-1}$, chart speed was 5 $mm \cdot min^{-1}$. The injected volume was 1 μl of aqueous solution of methanol, ethanol or acetone. For each solvent the standard curve was linear within the concentration range of 10–200 $\mu g \cdot ml^{-1}$.

TLC detection of picotamide

A solution of picotamide monohydrate (1 $mg \cdot ml^{-1}$) was applied and eluted with mobile phase 1. Under UV light a single spot with an R_f of 0.41 was located; the sensitivity limit was 0.1 μg.

HPLC determination of picotamide

Standard solutions of picotamide monohydrate in ethanol were prepared. These contained 1, 3, 5, 7 and 10 $mg \cdot ml^{-1}$, respectively. 1 μl of each solution was injected into the HPLC and the peak area measured (r.t. = 9.0) against concentration, thus obtaining a linear calibration graph which was used to quantify picotamide raw material (5 $mg \cdot ml^{-1}$ ethanolic solution).

GC determination of solvent impurities

0.1 g of picotamide monohydrate was suspended in 1 ml of water and 1 μl of the clear solution was injected into the GC. If the peaks of the acetone, methanol or ethanol appeared in the chromatogram with r.t. of 4.2., 8.6 and 10, respectively, then it was possible to assess their amounts by comparing the areas with those of the peaks obtained using standard solvents.

HPLC determination of 4-methoxyisophthalic acid (**VIII**)

A calibration curve was prepared by injecting 5 μl of 0.005, 0.01, 0.02, 0.03 and 0.04 $mg \cdot ml^{-1}$ standard solution of 4-methoxyisophthalic acid, dissolved in ethanol, into the HPLC. Under the above experimental conditions, except for the attenuation value which was brought up to A × 16, area peak and height values were obtained which were directly proportional to the concentration.

Results and Discussion

The HPLC method provided a precise and accurate picotamide determination in the concentration range 1–10 $mg \cdot ml^{-1}$. The calibration graph obtained was rectilinear ($y = 51.4x + 8.7$, $r = 0.9998$) with an average recovery ($n = 5$), at the 5 $mg \cdot ml^{-1}$ level, of 100.3 ± 1.2%. On one batch of picotamide monohydrate 10 analyses were carried out: the results obtained are summarized in Table 1.

TLC performed with mobile phase mixtures 3 and 4 (indicated above) allowed the detection of phenolic compounds **II, III** and **IV**. The location system was ferric chloride with R_f of 0.90, 0.55, 0.33 and 0.90, 0.74, 0.52, respectively. With the same eluents picotamide showed an R_f of 0 and 0.21, respectively. When instead, mixture 2 was used as eluent and UV light as detector, it was possible to reveal compounds **VII, XI** and **XII** with R_f of 0.86, 0.77 and 0.80, respectively, as well as picotamide ($R_f = 0.60$). When 500 μg of picotamide monohydrate (alcoholic solution) was applied, the TLC method was able to show single amounts of impurities in the order of 1 μg.

TABLE 1

HPLC analysis of picotamide (5 mg/ml ethanolic solution)

Sample	Recovery (%)
1	98.2
2	100.4
3	97.6
4	99.3
5	98.8
6	101.4
7	97.6
8	98.5
9	100.8
10	99.2
Average = 99.2%	
CV = 1.3%	

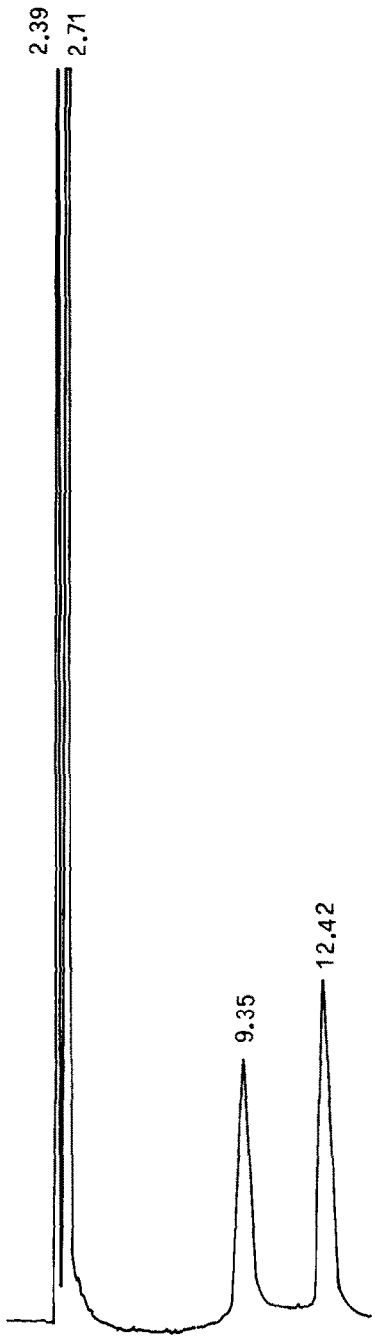

Fig. 2. HPLC analysis of a standard mixture. For chromatographic conditions see text. For key to compounds see Fig. 1. Key to peaks (r.t.): **VIII**, 2.39; **XI** and **XII**, 2.71; **X**, 9.35; **XIII**, 12.42.

The identification of compounds **VIII, XI, XII** and **XIII** was also possible with HPLC; when alcoholic solutions of single pure compounds were injected, under the experimental conditions described above, single peaks with r.t. of 2.36, 2.62, 2.69, 12.24, respectively, were observed, while picotamide (**X**) showed a single peak with an r.t. of 9.0.

Fig. 2 gives the chromatogram of a mixture of the above-mentioned 5 compounds submitted under the same chromatographic conditions. It may be noted that the r.t. of products **VIII, X** and **XIII** differ only slightly, while the separation of products **XI** and **XII** was not possible in that they showed only one peak with an r.t. of 2.71.

Of the above-mentioned potential impurities, particular attention was paid to 4-methoxyisophthalic acid (**VIII**), mainly because of its reported pharmacological activity (De Cunto et al., 1983). When concentration was plotted against peak area or height, a linear graph was obtained which allowed the HPLC determination of 0.01 μg of 4-methoxyisophthalic acid.

As regards the determination of organic solvent residues, the sensitivity of the method allows the measurement of methanol, ethanol or acetone at concentrations as low as 0.5 $\mu g \cdot ml^{-1}$.

The results of this study show that both TLC and HPLC are suitable methods for the qualitative and/or quantitative determination of picotamide raw material. Moreover, with TLC, impurities may be revealed at concentrations as low as 0.2% and with HPLC, starting from 0.1%. It is important to note that this method allows the simultaneous determination of the raw material and of any potential impurities. GC also allows the determination of any solvents present, at a concentration as low as 0.5 ppm. The analytical methods considered here may thus be usefully employed for the quality control of picotamide.

Acknowledgements

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