Journal of Chromatography, 146 (1978) 503—507
Biomedical Applications
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 212

Note

Gas chromatographic determination of phenazone derivatives in human plasma

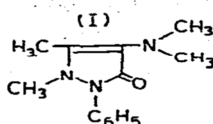
I. Aminophenazone

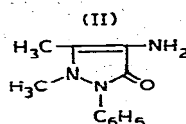
ANTOINE SIOUFI* and DANIELE COLUSSI

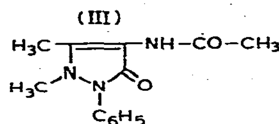
Ciba-Geigy, Centre de Recherche Biopharmaceutique, B.P. 308, 92506 Rueil-Malmaison Cedex (France)

(Received March 1st, 1978)

Aminophenazone (I) is an antipyretic, analgesic and anti-inflammatory drug used in various formulations:







Several methods for the assay of I, especially by gas chromatography (GC) have been described. Windorfer and Röttger [1] reported the GC assay of I in serum, down to 1 μg/ml, with docosane as internal standard, Lavene et al. [2] described a GC method for the assay of I down to 4 μg in 5 ml plasma or 20 ml urine, and of two of its metabolites together with caffeine and barbitone. Hexobarbitone was used as internal standard. The GC of I was carried out on silicone rubber columns without derivative formulation by Fuerst et al. [3]. Vesell et al. [4] determined I in plasma and saliva down to 2 μg/ml. Aminophenazone was extracted in chloroform containing antipyrine as internal standard.

None of these techniques is sufficiently sensitive to assay I after single-dose administration. This paper describes an improved procedure which permits determinations of I down to 0.1 μg/ml.

*To whom correspondence should be addressed.

EXPERIMENTAL

Chemicals and reagents

Aminophenazone was supplied by Ciba-Geigy (Basle, Switzerland). 4-Amino-antipyrine was purchased from Baker (Gross Gerau, G.F.R.) and 4-acetylamino-antipyrine from Aldrich (Beerse, Belgium). Buffer pH 12 (Titrisol; Merck, Darmstadt, G.F.R.) is prepared by diluting the contents of 4 vials in 500 ml water. Chloroform, carbon disulphide and isoamyl alcohol are of analytical grade (Merck). The carbon disulphide internal standard solution contains 50 μg/ml of heneicosane (C_{21}) [Kit No. 26A; Polyscience Corporation, Niles, Ill., U.S.A.].

Equipment

The tubes to be used for the evaporation of the solvent after extraction are previously treated to prevent adsorption. They are immersed for a few seconds in a silicone (Siliclad: Clay Adams, Parsippany, N.J., U.S.A.) bath [1% (v/v) aqueous solution], rinsed first with tap water and then demineralized water, and lastly dried at 100°.

A gas-chromatograph (Fractovap 2400 T; Carlo Erba), equipped with a flame-ionization detector is used. The peak areas are recorded by an electronic integrator (Infotronics, CRS 204). The column is operated at 215°, the injector at 250° and the manifold at 270°, with a nitrogen flow-rate of 40 ml/min. Glass columns are washed with 1.0 *N* hydrochloric acid, distilled water, acetone and benzene, then silanized with a 1% (v/v) solution of hexamethyl disilazane in benzene. After this treatment, the columns are washed again with benzene and dried at 100°. The column packing is 5% SE 30 on Chromosorb W, 80–100 mesh (Applied Science Labs, State College, Pa., U.S.A.).The filled column (2 m × 3 mm I.D.) is gradually heated up to 260°. The temperature is then increased 6–8 times from 150 to 250° in about half an hour and 20 μl of Silyl 8 (Pierce, Rockford, Ill., U.S.A.) is injected during every cycle.

Extraction

A 50-μl sample of the internal standard solution (50 μg/ml) is measured into a glass tube. The solution is taken to dryness under a nitrogen stream. A 1-ml volume of the sample, 2 ml of pH 12 buffer, 1 ml of 0.1 *N* NaOH and 5 ml of a 1% solution of isoamyl alcohol in chloroform are introduced into the tube, which is stoppered and shaken mechanically (Infors) for 20 min at 200 rpm and centrifuged at 4800 *g* for 15 min. The aqueous phase is pipetted off and discarded and an aliquot of the chloroform phase is transferred to a silicone-treated tube and taken to dryness under a nitrogen stream in a dry bath at 30°.

Gas chromatography

The dry residue is dissolved with 100 μl of carbon disulphide and the tube is shaken on a mixer (Vortex).

A 2-μl portion of the carbon disulphide solution is injected into the gas chromatograph by the solvent-flush technique. It is necessary to raise the oven temperature after 10 consecutive injections to 250° for half an hour to wash out plasma residues from the column.

The content of I is calculated from the peak-area ratio by reference to a cali-

bration curve. This curve is plotted on the basis of a 0.1N NaOH solution containing 50 μg/ml of I. Aliquots of this solution are transferred to tubes and plasma is added to yield plasma solutions containing 0.10—5.00 μg/ml.

RESULTS AND DISCUSSION

Precision and recovery

Table I gives the results obtained when the described procedure was applied to spiked plasma samples. Concentrations down to 100 ng/ml can be accurately determined. This sensitivity is not obtained when the glass tubes are not treated with silicone.

TABLE I

PRECISION AND RECOVERY OF THE DETERMINATION OF AMINOPHENAZONE APPLIED TO SPIKED HUMAN PLASMA SAMPLES

Amount added (ng/ml)	Amount found (ng/ml)	Mean	Precision reproducibility CV (%)	Recovery accuracy
100	78			78.8
100	94			94.0
100	83			83.0
100	85	86	10.6	85.0
100	77			77.0
100	100			100.0
200	189			94.5
200	192			96.0
200	203	189	6.4	101.5
200	194			97.0
200	171			85.5
500	482			96.4
500	533			106.6
500	522			104.4
500	436	476	9.6	87.2
500	461			92.2
500	419			83.8
1000	967			96.7
1000	958			95.8
1000	970	972	3.7	97.0
1000	1029			102.9
1000	990			99.0
1000	921			92.1
5000	4837			96.7
5000	4675			93.5
5000	4789			95.8
5000	4951	4920	4.6	99.0
5000	4931			98.6
5000	5338			106.8
			Mean	94.3 ± 8.2

Plasma interference

Fig. 1 shows the chromatograms of an extract of human plasma and of the same extract spiked with 4 μg of I and 2.5 μg of internal standard. There is no interference of the normal components of the plasma extract.

Metabolites

Several metabolites of I have been described by various investigators and reviewed recently by Van Ginneken [5] and Steltzer [6]. Most have been identified by thin-layer chromatography or combined gas—liquid chromatography—mass spectrometry in urine of animals and man. 4-Amino-antipyrine (II) and 4-acetylamino-antipyrine (III) are the main unconjugated metabolites described by Lavene et al. [2], Gradnik and Fleischmann [7], Momose and Suji [8], and Oehne and Schmid [9].

Plasma spiked with these two metabolites was processed under the conditions described for the assay of I. No interference from II or III was detected. The other known metabolites of I (4-methyl-amino-antipyrine, 4-hydroxy-antipyrine, rubazonic acid and methylamino-rubazonic acid) have been found in small amounts in urine, but they have not been reported in plasma. Therefore, the proposed method can be considered specific for the unchanged drug.

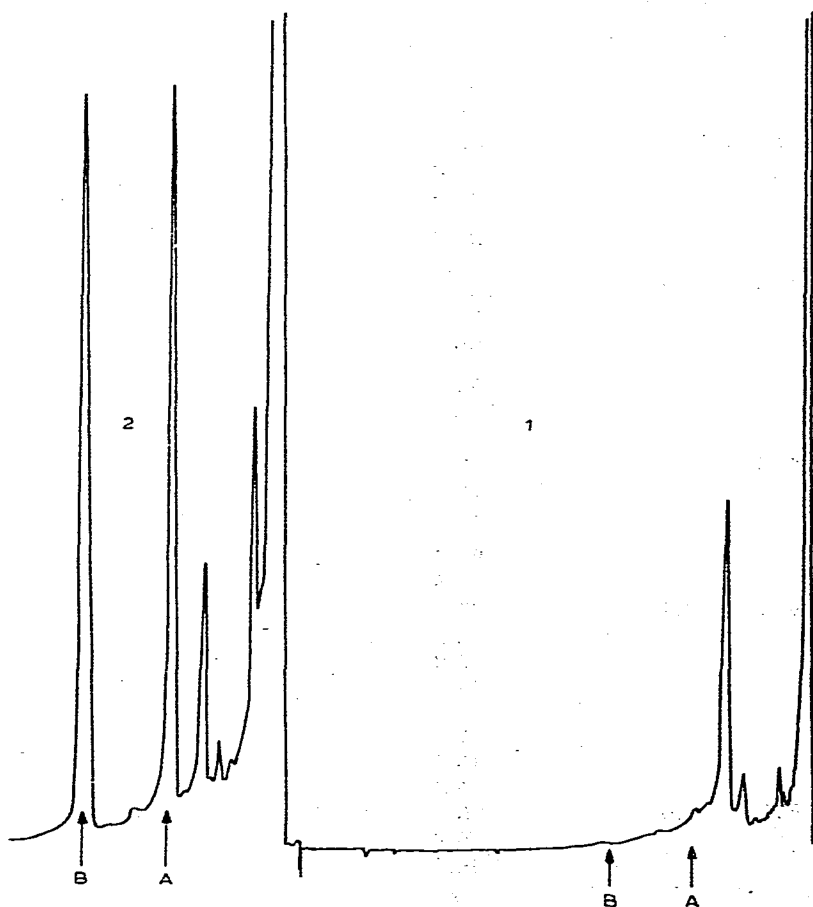


Fig. 1. Examples of chromatograms: 1 = human plasma blank; 2 = 4 μg/ml of aminophenazone (A) and 2.5 μg/ml of internal standard (B) in human plasma.

Human experiment

This method was applied to the determination of I in the plasma of one volunteer who had received one 300-mg tablet aminophenazone daily for three days. The results are shown in Table II. The plasma half-life of I was calculated from the data of the first day and found to be 2.0 h, in agreement with the value reported by Vesell et al. [4].

TABLE II

AMINOPHENAZONE PLASMA CONCENTRATIONS AFTER DAILY ADMINISTRATION OF 300 mg TO ONE VOLUNTEER FOR THREE DAYS

N.D. = not detectable.

Day of drug administration	Hours after administration	Aminophenazone plasma concentrations (μg/ml)
	0	N.D.
	2	3.3
1	4	1.3
	6	0.6
	8	0.3
	0	0.1
	2	3.9
3	4	1.1
	6	0.8

CONCLUSION

The proposed technique permits the rapid assay of aminophenazone in plasma with an adequate degree of accuracy and specificity. It is sufficiently sensitive to measure plasma levels after single-dose administration.

ACKNOWLEDGEMENTS

The authors are indebted to Dr. W. Theobald, Pharmacological Chemistry, Ciba-Geigy, Basle, for performing the clinical experiment.

REFERENCES

1 A. Windorfer, Jr. and H.J. Röttger, Arzneim.-Forsch., 24 (1974) 893.
2 D. Lavene, M. Guerret, H. Humbert and J.L. Kiger, Ann. Pharm. Fr., 32 (1974) 505.
3 W. Fuerst, H. Lauterbach, K.H. Daute and E. Klust, Zbl. Pharm. Pharmakother. Laboratoriumsdiagn., 109 (1970) 907.
4 E.S. Vesell, G.T. Passananti, P.A. Glenwright and B.H. Dvorchik, Clin. Pharmacol. Ther., 18 (1975) 259.
5 A.H. van Ginneken, Pharmacokinetics of Antipyretic and Anti-inflammatory Analgesics, Thesis 1976 (Stichting Studentenpers Nijmegen).
6 W. Stelzer, Inaugural dissertation, 1973, Institut für Rechtsmedizin der Universität, Erlangen, Nürnberg.
7 B. Gradnik and L. Fleischmann, Pharm. Acta Helv., 48 (1973) 144.
8 A. Momose and T. Suji, J. Pharm. Soc. Jap., 92 (1972) 187.
9 H. Oehne and E. Schmid, Arzneim.-Forsch., 22 (1972) 2115.