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THE PURITY OF PHENYLBUTAZONE RAW MATERIAL AND SOLID DOSAGE FORMS AS MONITORED BY GAS–LIQUID CHROMATOGRAPHY

J. R. WATSON, F. MATSUI, R. C. LAWRENCE AND P. M. J. McCONNELL
Research Laboratories, Health Protection Branch, Ottawa (Canada)
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SUMMARY

A simple gas–liquid chromatographic procedure for the estimation of intact phenylbutazone and of total degradation impurities in raw material and commercial dosage form samples is presented. Solutions are injected on to a phenylmethylsilicone column and the relevant peaks are quantitated by means of an electronic digital integrator with reference to an internal standard. The gas–liquid chromatographic method precludes many of the inherent disadvantages such as non-specificity and tediousness of other published procedures. It is applicable to the analysis of both official preparations and of other formulations containing buffering agents.

INTRODUCTION

Despite the multiplicity of methods available in the chemical literature for the analysis of phenylbutazone in biological media and in commercial dosage forms[1–10], there appears to be a dearth of specific techniques for the routine, precise measurement of the amount of undegraded phenylbutazone in the presence of its decomposition products. This is somewhat surprising, particularly in view of the reported instability of the drug in injectables, suppositories and tablet preparations under normal storage conditions[11,12]. The generally recurring limitations of non-specificity and/or tediousness of the pharmacopeial[13,14] and other published procedures clearly underscore the need to develop new methodology compatible with a rapid screening program for the assessment of the purity of phenylbutazone and its preparations.

The gas chromatographic procedure described here for the quantitation of phenylbutazone and for the sum total of its decomposition products in official and non-official preparations evolved from some rather fortuitous observations on the behaviour of phenylbutazone and its degradation products[15] when injected on to a phenylmethylsilicone column. While the former compound is eluted as a single symmetrical peak, under our conditions each of the latter* undergoes breakdown at the injection site to give a consistent retention time pattern of artifact peaks, the aggregate areas of which are proportional to the amount of degradation originally present in the formulation. The extent of deterioration can be monitored automatically by electronic integration of the relevant peaks with reference to a suitable internal standard.

* Courtesy of Geigy Pharmaceuticals.

The purpose of this investigation is to demonstrate the accuracy and specificity of the gas–liquid chromatographic (GLC) method by comparing the sum of the recoveries of phenylbutazone and its decomposition products with the results obtained using a direct but non-specific ultraviolet (UV) spectrophotometric procedure.

EXPERIMENTAL

Gas chromatographic system

The gas chromatograph (Bendix Series 2500 gas chromatograph), designed with four flame ionization detector units, two solid state electrometers and a multilinear automatic temperature programmer, was fitted with a 5% OV-7 (Pierce Chemical Company) on Gas-Chrom Q (Applied Science Laboratories) (100–120 mesh) U-shaped glass column (6 ft. × 6 mm O.D.) preconditioned at 265° for 24 h. The support was coated by means of a Hi-Eff fluidizer (Applied Science Laboratories), maintained at a temperature of 150° for 2 h with a suitable nitrogen flow to ensure gentle yet thorough drying of the packing material. Injection port and detector block temperatures were maintained at 230° and 240°, respectively. Gas flows were: nitrogen, 60 ml/min; hydrogen, 40 ml/min; air, 380 ml/min.

The detector signal was fed to a continuous balance dual pen, 1-mV recorder (Minneapolis-Honeywell, Elektronik 19 strip chart recorder) with variable chart speed set at 0.20 in. (0.508 cm)/min and connected to a fully automatic print-out electronic digital integrator (Kent, Model Chromalog 2) with a selected input signal range of 0–100 mV thus permitting a constant attenuation setting of 5×10^{-11} a.f.s.

Preparation of phenylbutazone–internal standard calibration curve

Materials. Phenylbutazone (Butazolidin), authentic material, was obtained from Geigy Pharmaceuticals and diphenyl phthalate (internal standard) (purity 97 % +), from Aldrich Chemical Company. The ethyl acetate used was A.C.S. grade.

Solutions. The internal standard was accurately weighed to contain about 2 mg/ml in ethyl acetate.

Preparation of mixtures and gas chromatography. Aliquots of approximately 20, 40, 80, 100, and 140 mg of pure phenylbutazone were accurately weighed into five separate 42-ml centrifuge tubes (Wilkens-Anderson Co., Chicago, Ill., Cat. No. 15846). To each aliquot was added: water (2 ml), concentrated HCl (0.25 ml) and ethyl acetate (10 ml). The mixtures were tumbled end-over-end at 100 r.p.m. for 15 min in a mechanical device designed to process fifteen tubes simultaneously, and then centrifuged for 2 min. In each case, a portion of the organic layer (2 ml) was diluted with the exact volume of solution equivalent to 4.00 mg of internal standard, dispensed from a 5-ml microburet (graduated in 0.01 ml). Two microliters of the final solution were injected into the gas chromatograph by means of a Hamilton microsyringe. The column temperature was kept isothermal at 190° for 10 min, then programmed at a rate of 7°/min to 225° and until complete elution of the internal standard (about 48 min). The area ratios of phenylbutazone to internal standard were plotted against the corresponding weight ratios in the mixtures.

Analysis of commercial dosage forms

Sampling. The formulations were sampled as follows: ten capsules were

emptied, their contents weighed and thoroughly mixed. In the case of the tablets, ten were selected at random, weighed and finely powdered.

Analysis by the GLC procedure. A quantity of the powder equivalent to about 100 mg of phenylbutazone was accurately weighed into a centrifuge tube and the drug extracted as described above. The upper layer of the centrifugate (2 ml) was diluted with the internal standard solution (equivalent to 4.00 mg) and the mixture chromatographed. The amount of phenylbutazone, χ (in mg), in the sample aliquot taken was computed from the relationship $\chi = 20(y/m)$ using an m value of 1.0736 and y as the experimental peak area ratio of phenylbutazone to internal standard. The percentages of phenylbutazone (retention time 31.68 min) values were calculated on the basis of the label claim. For the calculation of the amount of decomposition, the areas of the peaks at retention times of 3.96, 4.84, 6.60, 7.92 and 24.20 min were combined and the total weight χ^1 (in mg) of decomposition products in the aliquot taken was calculated using the relationship $\chi^1 = 20(y^1/m)$ and assuming equal detector response m for phenylbutazone and its degradation products. In this case, y^1 is the area ratio of the aggregate decomposition area to that of the internal standard. The results were expressed in terms of the label claim of phenylbutazone.

Analysis by the UV procedure. A 2-ml aliquot of the centrifugate obtained above was diluted to a 100-ml volume with chloroform. Five milliliters of this solution were evaporated to dryness and the residue taken up with 0.1 N NaOH to a final volume of 100 ml. The absorbance was measured on a suitable spectrophotometer at 264 nm against a standard solution prepared in the same manner. The results were expressed as percentages of label claim.

RESULTS

The calibration curve of phenylbutazone against internal standard is shown in Fig. 1. The weight ratios given are those calculated on the basis of the weight of

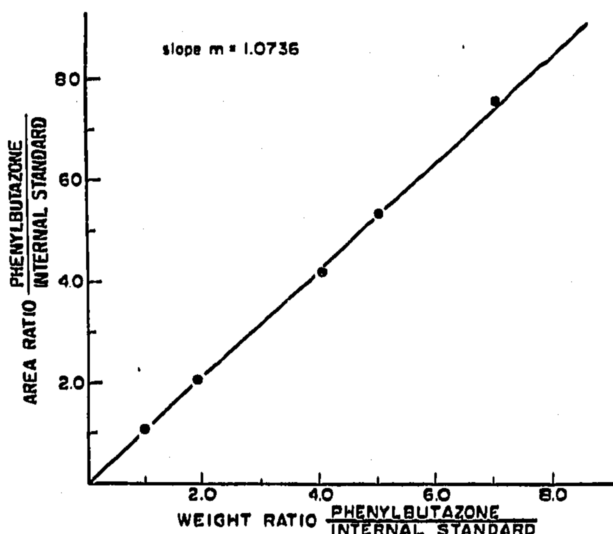

Fig. 1. Calibration curve of phenylbutazone *vs.* internal standard.

phenylbutazone in the solutions being chromatographed, *i.e.* one-fifth of the amount weighed out. Each of the five points on the line which passes through the origin represents the average of duplicate injections. The slope m (response factor) was calculated using the least squares equation to be 1.0736 with a coefficient of variation of $\pm$ 1.74%. The calibration was checked daily during the period of the investigation but the variation from the line never exceeded the mean slope error. It is important to select and optimize integrator parameters in order to achieve a calibration curve with a low coefficient of variation. Insufficient slope sensitivity, for example, can lead to significant underestimations in peak areas and appreciable deviation from the $y = mx$ situation. Because of the close chemical similarity of the decomposition products to phenylbutazone itself, they were assigned the same detector response factor as was measured for the parent drug. In practice, the areas due to the decomposition peaks at retention times of 3.96, 4.84, 6.60, 7.92, and 24.20 min were totaled and divided by the area of the internal standard peak to give a y^1 value which was converted to the corresponding χ^1 value using the response factor m of 1.0736.

The results of the analysis of six commercial formulations in tablet and capsule form are shown in Table I. All values are expressed as a percentage of label claim. In the UV procedure, any foreign species, as for example, the decomposition products, which absorb light energy at 264 nm, contribute to a net overestimation in phenylbutazone content. This non-specificity is precluded in the GLC procedure. On the basis of simple theoretical considerations, the sum (column 5) of the values for phenylbutazone (column 3) and total decomposition (column 4) should very nearly be that obtained by the UV procedure (column 6). To within experimental error, this agreement was achieved for the products examined.

TABLE I

ANALYSIS OF COMMERCIAL DOSAGE FORMS

Product number	*Dosage form*	*Found by GLC method (% label claim)*			*Found by UV method (% label claim)*
		Phenylbutazone	*Decomposition*	*Total*	
I	tablets[a]	101.78	0.15	101.93	101.39
II	capsules	81.90	4.38	86.28	85.80
III	tablets[a]	96.55	0.23	96.78	98.02
IV	tablets	95.32	0.57	95.89	96.62
V	capsules	88.28	3.50	91.78	90.05
VI	tablets	95.77	0	95.77	97.89

[a] Contain buffering agents.

DISCUSSION

In our experience the phenylmethylsilicone OV-7 has proven to be extremely useful in the GLC analysis of all types of pharmaceutical compounds. On a high-quality support, stationary phases of this type exhibit low bleed rates and generally require minimal conditioning time. With the appropriate temperature and gas flow

conditions, the large majority of currently used drugs are eluted from this column in a highly symmetrical fashion.* Diphenyl phthalate fulfilled all of the accepted criteria of a suitable internal standard: it is a stable compound not subject to thermal degradation or rearrangement; it had an FID response similar to the compounds being measured and was well resolved from the other peaks of interest. Under the injection port and column temperatures selected for this investigation, pure phenylbutazone (Fig. 2) passed through the column unchanged. This was confirmed by the addition of pure phenylbutazone to a degraded sample and subsequent quantitative recovery of the drug. Depending on the rate of heating, a slightly rising baseline (observed in the present case just following the isothermal phase) can be expected when temperature programming on a single channel, particularly when the electrometer is set at a low attenuation. However, as is usually the case, the small amount of bleed-off did not interfere with the quantitation of the pertinent peaks.

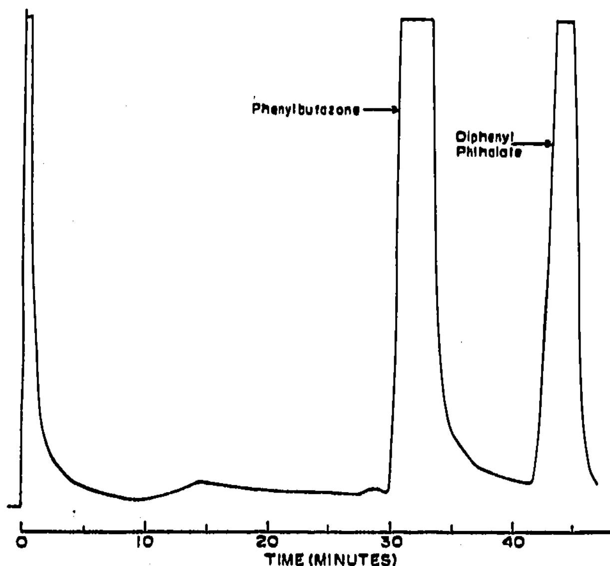

Fig. 2. Gas chromatogram of pure phenylbutazone and internal standard.

When a degraded sample of phenylbutazone, containing products of hydrolysis, oxidation or decarboxylation, is injected on to the column, a consistent pattern of "artifact" peaks is obtained at retention times of 3.96, 4.84, 6.60, 7.92 and at 24.20 min in instances of quite extensive deterioration. The presence of these peaks thus fortuitously serves as a monitor of phenylbutazone purity. A typical chromatogram of a decomposed sample is shown in Fig. 3. This product was a capsule preparation, the contents of which appeared to be quite discoloured when compared to similar formulations. Indeed, it was found by GLC to have a 4.38 % level of decomposition. Each of the six degradation products of phenylbutazone[15] obtained in pure form from the manufacturer was injected individually under the same temperature

* While ethyl acetate is commercially available in a highly pure state, in accordance with standard analytical practice each batch of the solvent used during the investigation was chromatographed as a blank to confirm the absence of any interfering materials.

program conditions as for phenylbutazone, and, in each case, at least two of the four artifact peaks which are eluted during the isothermal phase were observed. The peak at retention time of 3.96 min was common to all six compounds. The fact that no other peaks (other than those mentioned above) were noted suggests that complete breakdown of the compounds was occurring, probably at the injection port. It is interesting to note that azobenzene, a possible end-product of phenylbutazone degradation, is eluted as two peaks, the major one at a retention time of 3.96 min and a minor one at 7.92 min, apparently due to thermal equilibration to *cis* and *trans* isomers[11].

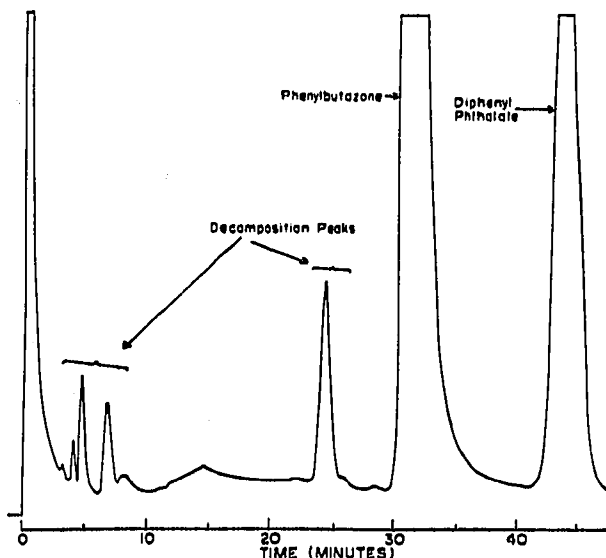

Fig. 3. Gas chromatogram of decomposed phenylbutazone and internal standard.

Official preparations do not normally present a problem with regard to the extraction of the drug. This is usually accomplished easily with the use of acetone or of sodium hydroxide solution. The situation with preparations formulated with buffering agents can, however, be quite different. Because of the lability of the C-4 hydrogen of phenylbutazone, compounds such as aluminium hydroxide or magnesium carbonate can very conceivably complex the drug as a salt and prevent its quantitative extraction from the dosage form. This, of course, dictated the choice of a strongly acidic medium for the complete dissolution of phenylbutazone from capsules and tablet matrices. Acidified ethyl acetate appeared to be a suitable extraction solvent for the majority of the commercial preparations examined.

CONCLUSION

Many of the published procedures for the analysis of phenylbutazone and its products demand time-consuming manipulations and most lack specificity for the pure drug in the presence of its degradation compounds. The GLC procedure described herein eliminates much of the tedium of the former methods and presupposes very

little expertise on the part of the analyst. Its outstanding advantage is that, if decomposition has occurred, it can be readily monitored and its extent measured without the prerequisite of having samples of pure degradation products available. The GLC procedure is, therefore, particularly feasible for medium or large-scale investigational programs where a reliable and rapid assessment of the quality of the formulation or of the raw material is required.

REFERENCES

1 R. Kalvoda and J. Zyka, *Chem. Listy*, 46 (1952) 57.
2 H. Hoehlein, *Pharmazie*, 21 (1966) 464.
3 M. Deffner and A. I. Deffner, *Chim. Anal.* (*Paris*), 40 (1958) 460; *C.A.*, 53 (1959) 10666.
4 A. I. Gengrinovitch and A. V. Serdeshnev, *Aptech. Delo*, 9 (1960) 13; *C.A.* 57 (1962) 1519.
5 R. K. Mitra and G. K. Ray, *Indian J. Pharm.*, 25 (1963) 262.
6 R. O. Searl and M. Pernarowski, *Can. Med. Ass. J.*, 96 (1967) 1515.
7 S. Ahuja, C. Spitzer and F. R. Brofazi, *Technicon Symp., Automation Anal. Chem., New York, October 2–4, 1967.*
8 J. E. Wallace, *J. Pharm. Sci.*, 57 (1968) 2053.
9 R. Perego, E. Martinelli and P. C. Vanoni, *J. Chromatogr.*, 54 (1971) 280.
10 M. Theodor, *Pharm. Ztg.*, 115 (1970) 139.
11 E. Pawelczyk and R. Wachowiak, *Acta Pol. Pharm.*, 26 (1969) 425.
12 H. D. Beckstead, K. K. Kaistha and S. J. Smith, *J. Pharm. Sci.*, 57 (1968) 1952.
13 *United States Pharmacopeia*, Mack, Easton, Pa., 18th Rev., 1970, p. 497.
14 *British Pharmacopoeia 1968*, Pharmaceutical Press, London, 1968, p. 762.
15 G. A. Neville and D. V. C. Awang, *Org. Magn. Resonance*, 2 (1970) 341.