*Journal of Pharmaceutical & Biomedical Analysis*
Vol. 9, No. 1, pp. 87–89, 1991
Printed in Great Britain

0731–7085/91 $3.00 + 0.00
© 1991 Pergamon Press plc

*Short Communication*

# Quantitative determination of analgesic mixture of phenazone, phenacetin and caffeine in the presence of some of their degradation products

M. EL SADEK,*† A. EL SHANAWANY,† A. ABOUL KHIER† and G. RÜCKER‡

† *Faculty of Pharmacy, Zagazig University, Zagazig, Egypt*
‡ *Institute of Pharmacy, Bonn University, Bonn, FRG*

**Keywords**: *Phenazone, phenacetin and caffeine mixture; HPLC method.*

## Introduction

The mixture containing phenazone, phenacetin and caffeine is effective as an antipyretic analgesic anti-inflammatory. The ingredients of the mixture are determined separately or in binary forms by spectrophotometry [1], titrimetry [2], thin layer chromatography [3], and high-performance liquid chromatography (HPLC) [4–6].

## Experimental

*Instrument*

A HPLC apparatus is constructed from Water Associates 600, a solvent system, connected with a fixed wavelength UV detector (Water Associates 440), recorder (Servagor 220, Austria) and integrator (Hewlett–Packard 3390A).

*Chromatographic conditions*

The Lichrosorb Rp-8 column (7 μm) used was from Hibar Merck. The mobile phase was prepared by dissolving 0.866 g $KH_2PO_4$ in a mixture of 630 ml water and 370 ml methanol, and the solution adjusted to pH 4 by the addition of phosphoric acid. The flow rate was 1.5 ml $min^{-1}$ and the detector sensitivity was 0.2 a.u.f.s. at 254. The integrator used had a chart speed of 0.5 cm $min^{-1}$ and an attenuation of 2. All measurements were carried out at room temperature.

*Materials*

All chemicals and reagents were of HPLC grade. Phenazone and *p*-aminosalicylic acid were obtained from Aldrich Chemie (FRG); phenacetin was from Merck (FRG) and acetanilid was from Hoechst (FRG). *p*-Chloroacetanilide was synthesized and confirmed for its identity and purity [7]. Coffeemed tablets were obtained from Herbert Passauer (FRG). All stock solutions were prepared by dissolving 250 mg/50 ml for *p*-aminosalicylic acid, 200 mg/50 ml for caffeine, 100 mg/50 ml for phenazone and 150 mg/50 ml for phenacetin.

*Procedures*

*(A) Pure authentic samples.* A calibration curve for each component is performed by transferring different volumes of stock solutions into a 10 ml calibrated flask, adding 1 ml stock internal standard to each flask, then completing to volume with methanol–water (1:1). A 1 μl aliquot from each concentration is adjusted into the chromatograph under the described conditions, and the area ratios are integrated and plotted against concentration.

*(B) Authentic mixture.* Different aliquots from the authentic mixture stock solution

* Author to whom correspondence should be addressed.

within the range recorded in the calibration are transferred into a 10-ml calibrated flask and completed as described under procedure (A).

*(C) Commercial dosage forms.* Twenty tablets are weighed and the average weight of one tablet is determined. The tablets are finely powdered. A weight equivalent of one tablet is transferred into a 50-ml calibrated flask, and approximately 40 ml of methanol–water mixture added. The mixture is deareated by sonication for 10 min, then completed to volume. The mixture is filtered, and the first portion of the filtrate rejected. Different aliquots are transferred into a 10-ml calibration flask and completed as described under procedure (A).

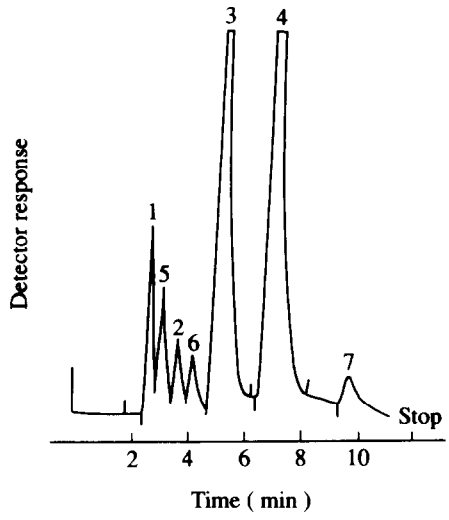


**Figure 1**
HPLC of authentic mixture containing *p*-aminosalicylic acid 0.1 μg (1), caffeine 0.1 μg (2), phenazone 0.5 μg (3), and phenacetin 0.4 μg (4), spiked with *p*-phenetidine 0.001 μg (5), acetanilid 0.01 μg (6), and *p*-chloroacetanilid 0.01 μg (7). Flow rate: 1.5 ml $min^{-1}$. Mobile phase: 37% methanol–0.01 M $KH_2PO_4$, pH 4.

## Results and Discussion

In the suggested method, methanol–water (63:37) is recommended as the most suitable mobile phase. Separation of the components was found to be effected by changing the pH of the mobile phase. This can be attributed to the fact that, decrease in pH decreases the ionization of weak acids, for instance phenazone and phenacetin cause increases in their retention times and vice versa regarding weak bases, e.g. caffeine. pH 4 was found to be the most effective pH for separation within considerable retention times.

Under the recommended chromatographic conditions, phenazone, phenacetin, caffeine and *p*-aminosalicylic acid as internal standard could be separated without interference from each other or from acetanilide, *p*-phenatidine and *p*-chloroacetanilide, which may be present as impurities or degradation products in commercial tablet form (Fig. 1).

The UV spectra of each component dissolved in the recommended mobile phase have been studied. It was found that a fixed wavelength at 254 nm had a suitable extinction coefficient, $E^{1\%}_{1cm}$, for caffeine, phenazone, phenacetin and *p*-aminosalicylic acid of 462, 673, 903 and 664, respectively. Therefore, the detector was set at 0.2 a.u.f.s.

Different concentrations of each constituent, in the presence of internal standard, were chromatogramed. The peak area ratio for each constituent to peak area of internal standard was calculated for each chromatogram and plotted against concentration. The relationship is linear within the concentration range 0.05–3.6 μg $ml^{-1}$ for caffeine, 0.1–2.8 μg $ml^{-1}$ for phenazone and 0.3–4 μg $ml^{-1}$ for phenacetin.

Regression analysis of the data for each component gave the slope, intercept and correlation coefficient for each calibration curve (Table 1).

The validity of the listed regression equation was tested by the assay of the authentic mixtures containing known quantities of phenazone, phenacetin and caffeine in a ratio equal to that found in commercial dosage forms (Table 2). The results showed good accuracy

**Table 1**
Regression analysis

| Drug | Regression equation | Correlation coefficient |
|---|---|---|
| Caffeine | $Y = 1.116X - 0.035$ | $r = 0.9994$ |
| Phenazone | $Y = 2.394X - 0.030$ | $r = 0.9990$ |
| Phenacetin | $Y = 3.395X - 0.147$ | $r = 0.9995$ |

**Table 2**
Analysis of authentic mixture of phenazone, caffeine and phenacetin using the proposed HPLC method

| Caffeine | | | Phenazone | | | Phenacetin | | |
|---|---|---|---|---|---|---|---|---|
| Amount added (mg ml$^{-1}$) | Amount found (mg ml$^{-1}$) | Recovery (%) | Amount added (mg ml$^{-1}$) | Amount found (mg ml$^{-1}$) | Recovery (%) | Amount added (mg ml$^{-1}$) | Amount found (mg ml$^{-1}$) | Recovery* (%) |
| 0.10 | 0.101 | 101.14 | 0.50 | 0.497 | 99.43 | 0.4 | 0.42 | 105.08† |
| 0.20 | 0.199 | 99.70 | 1.00 | 0.978 | 97.82 | 0.8 | 0.79 | 98.20 |
| 0.25 | 0.255 | 102.07 | 1.25 | 1.210 | 96.85 | 1.0 | 0.98 | 98.00 |
| 0.30 | 0.305 | 101.85 | 1.50 | 1.485 | 99.80 | 1.4 | 1.40 | 100.20 |
| 0.35 | 0.356 | 99.11 | 1.75 | 1.775 | 101.43 | 1.6 | 1.55 | 96.59 |
| 0.40 | 0.402 | 100.55 | 2.00 | 1.988 | 99.41 | 2.0 | 1.99 | 99.59 |
| 0.50 | 0.502 | 100.42 | 2.50 | 2.469 | 100.53 | | | |
| Mean | | 100.69 | | 99.32 | | | 98.53 | |
| SD | | 1.08 | | 1.56 | | | 1.25 | |
| RSD | | 1.07 | | 1.57 | | | 1.26 | |

*The result must be rejected according to the rejection rule.
†Average of five experiments.

**Table 3**
Analysis of Coffeemed tablets using the proposed HPLC method

| Caffeine | | | Antipyrine | | | Phenacetin | | |
|---|---|---|---|---|---|---|---|---|
| Amount claimed (mg ml$^{-1}$) | Amount found (mg ml$^{-1}$) | Recovery* (%) | Amount claimed (mg ml$^{-1}$) | Amount found (mg ml$^{-1}$) | Recovery* (%) | Amount claimed (mg ml$^{-1}$) | Amount found (mg ml$^{-1}$) | Recovery* (%) |
| 0.1 | 0.099 | 98.92 | 0.50 | 0.505 | 101.10 | 0.4 | 0.429 | 107.2 |
| 0.2 | 0.199 | 99.83 | 1.00 | 0.998 | 99.84 | 0.8 | 0.796 | 99.56 |
| 0.3 | 0.296 | 98.93 | 1.50 | 1.514 | 100.97 | 1.2 | 1.22 | 101.5 |
| 0.4 | 0.404 | 101.16 | 2.00 | 2.02 | 101.24 | 1.6 | 1.61 | 103.2 |
| 0.5 | 0.500 | 100.00 | 2.50 | 2.56 | 102.42 | 2.0 | 2.11 | 101.9 |
| | | | 2.75 | 2.74 | 99.79 | 2.2 | 2.24 | 98.7 |
| | | | | | | 2.4 | 2.37 | |
| Mean | | 99.77 | | 100.89 | | | 101.79 | |
| SD | | 0.93 | | 0.98 | | | 2.83 | |
| RSD | | 0.93 | | 0.97 | | | 2.78 | |

*Average of five experiments.

(as shown by the percentage recovery: 100.69 for caffeine, 99.32 for phenazone and 98.53 for phenacetin). The proposed method was applied to assay commercial tablets for the three components. Results show good accuracy and precision (Table 3).

The method is highly sensitive and time saving and can be used in the quality control of analgesic tablets containing this ternary mixture and tablets of similar composition. Also, impurities — which may be present — are detected with efficiency using the proposed method.

## References

[1] D. Bones, *J. Am. Pharm. Assoc. Sci.* **43**, 580 (1954).
[2] E.G. Wollish, R.J. Colarusso, C.V. Pifer and M. Schmall, *Anal. Chem.* **26**, 1753 (1954).
[3] H.W. Dibbern and G. Scholz, *Arch. Pharm.* **298**, 175 (1965).
[4] S. Ebel and G. Herold, *Chromatographia* **8**, 596 (1975).
[5] P. Guinebault and M. Broquarie, *J. Chromatogr.* **217**, 508 (1981).
[6] M.G. Quaglia, P. Mazzes and A. Lopez, *J. Pharm. Biomed. Anal.* **3**, 187 (1985).
[7] M.H. Broyles and W.K. Easley, *J. Org. Chem.* **25**, 2233 (1960).

[*Received for review 10 August 1989*]