

High Performance Liquid Chromatographic Method for the Simultaneous Determination of Acetaminophen and Acetophenetidine in Formulations

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Abstract

A simple and accurate HPLC method has been developed for the determination of acetaminophen and acetophenetidine in pharmaceutical preparations. Acetaminophen and acetophenetidine were analyzed simultaneously. Commercial pharmaceutical tablets were analyzed by the HPLC method and the concentration of each component was calculated from the regression equation. Calibration graphs were valid in the range 1-80 $\mu\text{g}\cdot\text{ml}^{-1}$ for acetaminophen and 2-40 $\mu\text{g}\cdot\text{ml}^{-1}$ for acetophenetidine. The results obtained by the proposed method were compared successfully with the results of the official method.

Introduction

Paracetamol has been widely used as an analgesic and antipyretic drug. In 1887 phenacetin was found to have analgesic properties, but in 1949 phenacetin's metabolite, acetaminophen, was found to be the true pain reliever. However, phenacetin still remains on the market in many combined formulations. Various simultaneous methods for the determination of paracetamol with other components have appeared in the literature, including chromatographic methods (Veerabhadrao *et al.*, 1987; Dinc, 1999; Argekar *et al.*, 1999; Akhtar *et al.*, 1994), which have been demonstrated for pharmaceutical preparations. HPLC methods for simultaneous determination of paracetamol in combination with other drugs such as methocarbamol (Erram and Tipnis 1993), caffeine and antipyrine (Yang, 1992), have been developed. A spectrophotometric method has been reported for the determination of paracetamol and phenacetin (Nagaraja *et al.*, 1998) as the hydrolysis products resulting from the reaction of paracetamol and phenacetin with sodium 1,2-naphthoquinone and cetyltrimethyl ammonium bromide. Paracetamol was monitored at 570 nm and phenacetin at 500 nm. Simultaneous determination methods of paracetamol and phenacetin in formulations have not been reported in the literature. Phenacetin is converted to paracetamol *in vivo*. Paracetamol has proven to be as effective as phenacetin but less toxic. Therefore, a simultaneous assay of both drugs in combination was of great interest. In the present communication, we developed a simple HPLC method for the simultaneous determination of acetaminophen and phenacetin in combined pharmaceutical formulations.

Materials and Methods

Apparatus: The HPLC equipment consisting of the following: A LC-6A liquid chromatograph (Shimadzu); A CTO-10A column oven equipped with a Rheody valve 20 μl sample injection loop (Shimadzu); A SPD-6AV UV-visible detector (Shimadzu); A C-R3A chromatopac integrator (Shimadzu); A D-2500 chromato-integrator (Hitachi) and Shim-Pack IC-A3 (s) column (Shimadzu).

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HPLC Conditions: The mobile phase was delivered at a constant flow rate and pressure (0.2 ml/min and 100kgf/cm², respectively) by isocratic pump and the eluent monitored at 260 nm. Separation was performed by means of Shim-Pack IC-A3 (s) column. The mobile phase was phthalic acid (0.9mM) and the pH adjusted to 4.6 with sodium hydroxide 0.1M. Flow rate of 0.2 ml/min was maintained at a temperature of 40°C.

Materials: Acetaminophen (Wako Pure Chemical Industries, Ltd, Japan) phenacetin (Aldrich Chemical Company, USA) and phthalic acid (Kato Chemical Co. Inc, Japan) and all the other chemicals used were of analytical reagent grade. Milli Q water was used in all the experiments.

Preparation of stock solution: Stock solution of acetaminophen (1mg.ml⁻¹) and phenacetin (1mg.ml⁻¹) were prepared with the mobile phase. A synthetic mixture was prepared by mixing paracetamol (PAR) and phenacetin(PHE) in the concentration range of 1-80 µg.ml⁻¹ and 2-40µg.ml⁻¹ of PAR and PHE, respectively. This solution was filtered through a 0.45µm membrane and degassed in a sonication water bath (Sharp UT-105) before injection into the chromatograph.

Calibration curves: Volumes of 0.01-0.8 ml of PAR and 0.02-0.4 ml of PHE were accurately transferred into 10 ml volumetric flasks and diluted to the mark with the mobile phase. 10µl of each concentration was injected into the column and the calibration curves were constructed by plotting the drug concentration against peak area.

Assay of pharmaceutical preparations: 15 tablets (commercial or laboratory made) were finely ground into a powder and weighed. Some ingredients such as starch, glucose, magnesium stearate, talc, sucrose, lactose, were mixed with the tablets powder. An equivalent amount of 100 mg of paracetamol and phenacetin were transferred into a 100 ml volumetric flask and dissolved within the mobile phase, stirred thoroughly and filtered. The recommended procedures were followed as for the determination of PAR and PHE.

Result and Discussions

Phenacetin is metabolized to acetaminophen. Which is further metabolised by enzymes of the cytochrome P-450 series to intermediate products, non reactive glucuronides and sulfates (which are conjugated and eliminated in the urine) and reactive metabolites; which are metabolized with glutathione to non toxic mercapturic acid which is eliminated. Therefore, it is important to develop a simultaneous separation and determination of paracetamol and phenacetin from pharmaceutical formulations.

Chromatography: The mobile phase (phthalic acid at pH 4.6) resolved paracetamol and phenacetin efficiently. The optimum pH adopted for the mobile phase was 4.6, since it achieves simultaneous analysis of paracetamol and it is metabolite at the same time without interfering or overlapping with each other. The maximum absorption of paracetamol and phenacetin was 260 nm, therefore this wavelength was used through out the analysis. The retention time (min) values were 7.78 and 5.25 for paracetamol and phenacetin, respectively. The total time for analysis was less than 8 min. Figure shows the chromatogram of authentic mixture containing paracetamol and phenacetin.

Linearity of the calibration curve: Table 1 shows the data of the calibration curve for the standard drug solutions. Plot of concentration of PAR and PHE against peak area were found to be linear in the range 1-80 µg.ml⁻¹ and 2-40 µg.ml⁻¹ for PAR and PHE, respectively.

Table 1. Calibration results for the paracetamol and phenacetin (n=5).

Compounds	Concentration range (µg.ml ⁻¹)	Slope	Intercept	Correlation coefficient (r ²)
Paracetamol	1-80	13261	8693.9	0.998
Phenacetin	2-40	71.87	3.72	0.9995

The limit of detection (LOD) and determination (LOD') for PAR and PHE were calculated by the following equation: $LOD = 3 \times \sigma/S$ and $LOD' = 10 \times \sigma/S$, where σ , the noise estimated in the standard deviation of response of blank samples ($n = 10$) and S is the slope of the corresponding calibration curve. Therefore, LOD and LOD' were found to be 1.9 ng.ml^{-1} and 9.7 ng.ml^{-1} for PAR and PHE, respectively.

Accuracy: The accuracy of the recommended procedure was checked and confirmed by the recovery test experiments. Tablet samples were added to three different concentrations of standard ($n = 5$). The recovery rates were calculated and found to range between 97.98 to 100.7% for PAR and 98.32 to 100.1% for PHE, respectively. RSDs ($n = 5$) ranged from 0.63-2.1% for PAR and 0.42-1.82% for PHE, respectively.

Interference: The effect of various compounds on the determination were studied. Compounds such as indomethacin, chlorpheniramine maleate, and ascorbic acid, were found not to interfere the determination. Moreover, Analgin, caffeine, diclofenac sodium, ibuprofen and chlorzoxazone were also found not to interfere with the determination.

Application: The proposed method was applied to the assay of formulation containing PAR and PHE. PHE was prepared in the laboratory, since it was not available in the market. The results obtained were compared successfully with the official method (BPXXII, 1993) and were satisfactory and showed that the method is applicable to the determination of PAR and PHE in pharmaceutical preparations (Table 2).

Table 2. Results of the determination of paracetamol and phenacetin in pharmaceutical preparations by the proposed HPLC method and official method.

Products*	Nominal composition (mg)	% Recovery, \pm RSD**	
		Proposed method	Official method ***
Paramet (Wallace)	500 paracetamol 5 metachlopramide	99.8 \pm 1.02	99.3 \pm 1.2
Saridon (Roche)	250 paracetamol 150 prophenazone 50 caffeine	100.8 \pm 0.86	100.2 \pm 0.83
Diclogesic (Torrent)	500 paracetamol 50 diclofenac sodium	101.2 \pm 0.38	99.8 \pm 0.51
Spandret (American Remed)	300 paracetamol 250 chlorzoxazone 400 ibuprofen	99.9 \pm 0.48	99.6 \pm 0.63
Dolopar	250 paracetamol 250 analgin 250 cafeine	101.0 \pm 0.32	100.4 \pm 0.82
Phenacetin (Laboratory made)	500	102 \pm 0.56	-----

* Trade mark ** Average of five determinations *** Reference 10.

Conclusions: The HPLC method is recommended for the simultaneous determination of paracetamol and phenacetin in tablets. The method proposed is simple, sensitive and selective, therefore, it is convenient for routine quality control analysis.

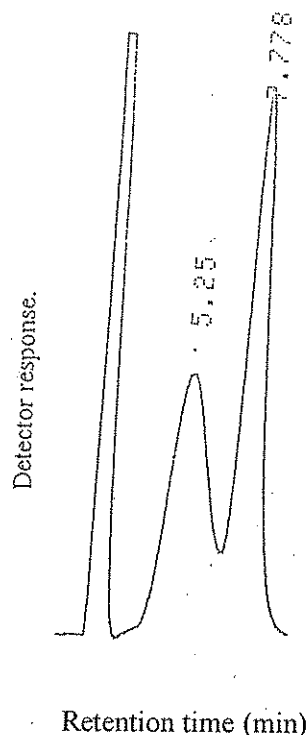


Figure 1
Chromatogram of authentic mixture containing (1) acetophenetidine (5.25 min); (2) Acetaminophen (7.78 min).

References

- Ahktar, M.J., Khan, S. and Hafiz M. (1994). Determination of ampicillin in human plasma by high performance liquid chromatography using ultra-violet detection. *J. Pharm. Biomed. Anal.* 12: 379-382.
- Argekar, A.P. and Sawat, J.G. (1999). Simultaneous determination of paracetamol and mefenamic acid in tablets by HPLC. *J. Planar Chrom.* 12: 361- 364.
- British Pharmacopoeia XXII, Office of The British Pharmacopoeia Commission, London p.483, 1993.
- Dinc, E. (1999). A comparative study of ratio spectra derivative spectrophotometry, Vierordt's method and high performance liquid chromatography applied to the simultaneous analysis of caffeine and paracetamol in tablets. *J. Pharm. Biomed. Anal.* 21: 723-730.
- Erram, S.V. and Tipnis, H.P. (1993). Simultaneous estimation of methocarbamol and paracetamol from single and combined dosage tablets by RP [Reversed-phase] HPLC. *Indian Drugs* 30: 116-119.
- Nagaraja, P., Murthy, K.C.S. and Rangappa, K.S. (1998). Spectrophotometric method for the determination of paracetamol and phenacetin. *J. Pharm. Biomed. Anal.* 17: 501- 506.
- Veerabhadrarao, M., Narayan, M.S. and Kapur O. (1987). Reverse-phase liquid chromatographic determination of some food additives. *J. Assoc. off. Anal. Chem.* 70: 578-582.
- Yang, F. (1992). Determination of contents of the three components in chlorpheniramine maleate tablets by high-performance liquid chromatography. *Yaowu Fenxi Zazhi* 12: 362-365.

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