RP-LC DETERMINATION OF ASCORBIC ACID, PARACETAMOL AND CAFFEINE IN MULTICOMPONENT ANTI-COLD PREPARATION

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ABSTRACT

An RP-LC method for the simultaneous determination of ascorbic acid, paracetamol and caffeine in a tablet matrix is described. The mobile phase was a mixture of methanol-NaH_2PO_4 (17:83 pH 4.25 ± 0.05) and stationary phase Aquapore RP-300. Total chromatographic time was approximately 8 minutes and the method found to be cost effective. The method was used to analyze some brands of multi-component anti cold preparations on the local market. Results were validated using the standard UV spectrophotometric methods for the assay of the individual components of these drugs.

Key Words: *Ascorbic acid, Paracetamol, caffeine, benzoic acid, RP-LC*

INTRODUCTION

The common cold is believed to be one of the most frequently presented conditions in the Hospitals, Clinics and Pharmacies [Henila, 1994]. As a result of trade liberalization and self-medication, many brands of multi-component anti cold preparations have appeared on the local market with diverse countries of origin. Cold preparations are usually complex formulations containing several active ingredients [Ovesen, 1984], methods available for assay most often involve extraction of the individual active ingredients [Sethi, 1993; British Pharmacopoeia, 1993].

The current study is aimed at designing an HPLC method that is cheap, fast and flexible. The method employs the use of a methanol based mobile phase, a reverse phase column and isocratic mode of elution. Chromatography was carried out at ambient temperature. Quantification was achieved using the method of internal calibration with benzoic acid serving as internal standard.

EXPERIMENTAL

Chemicals

Sodium thiosulphate BDH Analar grade, L-Ascorbic Acid (Weisheng Pharmaceuticals Company Limited, China, Batch number 9907032), Paracetamol powder (Weisheng Pharmaceuticals Company Limited, China, Batch number 980745), Caffeine anhydrous powder (Weisheng Pharmaceuticals Company Limited, China, Batch number 990756), Ammonium molybdate (BDH Analar grade), Sodium dihydrogen sulphate (BDH Analar grade), Methanol (BDH HPLC grade), Sulphuric acid (BDH Analar grade), Phosphoric acid (BDH Analar grade), Samples A_1, A_2, A_3, A_4. (BDH Analar grade).

Instrumentation

For isocratic elution a SHIMADZU analytical pump (LC-10AS) was used in conjunction with a Rheodyne 8125-095-syringe loading sample injector. A SHIMADZU UV/Visible detector (SPD-10A/10AV) was used to monitor the column eluate. For data analysis peak integration was performed using a SHIMADZU CR501 Chromatopac. UV analysis was done using a CECIL 8000 UV spectrophotometer.

Reagent Preparation

Solvent: 0.4g of sodium thiosulphate was weighed and dissolved in sufficient distilled water; the solution was transferred into a 1000ml volumetric flask and made to mark. The solution was used as solvent for dissolution.

Preparation of Calibration Solutions

A quantity of pure paracetamol (2g), ascorbic acid (0.2g), and caffeine anhydrous (0.12g) were weighed to mimic tablet concentrations. The three components were transferred into a 200ml volumetric flask and dissolved with sufficient 0.04%$^w/_v$ $Na_2S_2O_3$ by mechanically shaking for 15 minutes. This was made to the 200ml mark with more 0.04%$^w/_v$ $Na_2S_2O_3$ solution.

20ml of this was transferred into a 200ml volumetric flask and made up to mark. Aliquots of 5ml, 15ml, 25ml and 35ml were transferred into four 50ml volumetric flask and made to the mark with 0.04%$^{w}/_{v}$ $Na_2S_2O_3$. Thus the concentrations of Ascorbic acid were 10μg/ml, 30μg/ml, 50μg/ml, 70μg/ml and 100μg/ml; those for Paracetamol were 100μg/ml, 300μg/ml, 500μg/ml, 700μg/ml and 1000μg/ml and Caffeine were 6μg/ml, 18μg/ml, 30μg/ml, 42μg/ml and 60μg/ml in the respective calibration solutions.

Preparation of Test Preparation

20 tablets of the sample were weighed and powdered. A quantity of the powder equivalent to 500mg paracetamol, 50mg ascorbic acid and 30mg caffeine was weighed and transferred into a 1000ml volumetric flask and shaken mechanically with sufficient 0.04%$^{w}/_{v}$ $Na_2S_2O_3$ for 15 minutes. It was made up to mark with more 0.04%$^{w}/_{v}$ $Na_2S_2O_3$ solution. This was filtered using filter paper and 10ml of the resulting solution was diluted in a 1000ml volumetric flask such that the concentration of Ascorbic acid, Caffeine and Paracetamol were 50μg/ml, 30μg/ml and 500μg/ml, respectively.

Procedure for Analysis

40ml each of the calibration solutions was thoroughly mixed with 18ml of internal standard and 20μl of the mixture introduced unto the column by means of a rheodyne. The peak areas of the components were then measured. The above was repeated for the test solutions.

0.05M NaH_2PO_4 Buffer

7.8005g of NaH_2PO_4 was weighed and dissolved in sufficient distilled water in a 1000ml volumetric flask and made to mark. The pH of the solution was adjusted using Phosphoric acid. The solution was thoroughly filtered through 0.45μm membrane filter.

Mobile Phase

A mixture of methanol and Phosphate buffer varying proportions and pH were tried. It was detected that a mixture of methanol and 0.05M NaH_2PO_4, pH 2 in a proportion 17:83 gave different retention times for ascorbic acid, paracetamol and caffeine.

Preparation of Internal Standard

0.1% solutions of Chloroquine Sulphate, Diphenhydramine HCl, Ephedrine HCl and benzoic acid were prepared in 0.04%$^{w}/_{v}$ $Na_2S_2O_3$ solution and their retention times determined. Benzoic acid was found to have the best retention time to serve as internal standard. It was detected that the retention time of benzoic acid varied with pH of mobile phase. A pH of 4.25 was found to decrease considerably the chromatographic time. This was selected as the pH for analysis.

Chromatographic Conditions

- Stationary phase Aquapore RP 300,C_8 (250mm x 4.6mm i.d).
- The flow rate of mobile phase was 1.5ml/min.
- The effluent from the column was monitored at a wavelength of 273nm and 0.3 absorbance units full scale (AUFS).
- Chromatography was carried out at ambient temperature.

Stability Testing of Ascorbic Acid

40ml of the calibration solution containing 50μg/ml ascorbic acid, 500μg/ml paracetamol and 30μg/ml caffeine was mixed with 18ml of internal standard. 20μg/ml of the solution was introduced unto the column immediately. The respective peak area ratios were calculated. The administration was repeated hourly for six hours. The solution was kept and daily injections done for five days. The procedure was carried out simultaneously for similar solution in distilled water as control.

Limit of Detection

A solution of a mixture of 500mg of paracetamol, 50mg of ascorbic acid and 30mg of caffeine was prepared in 0.04%$^{w}/_{v}$ $Na_2S_2O_3$ solution. A stepwise dilution of this solution by a factor of 10 was made. Six replicates of each concentration were analysed to determine the limit of detection.

3 Results

1. Ascorbic acid 2. Paracetamol 3. Caffeine 4. Benzoic acid

pH 2 pH 4 pH 4.25 pH4.5

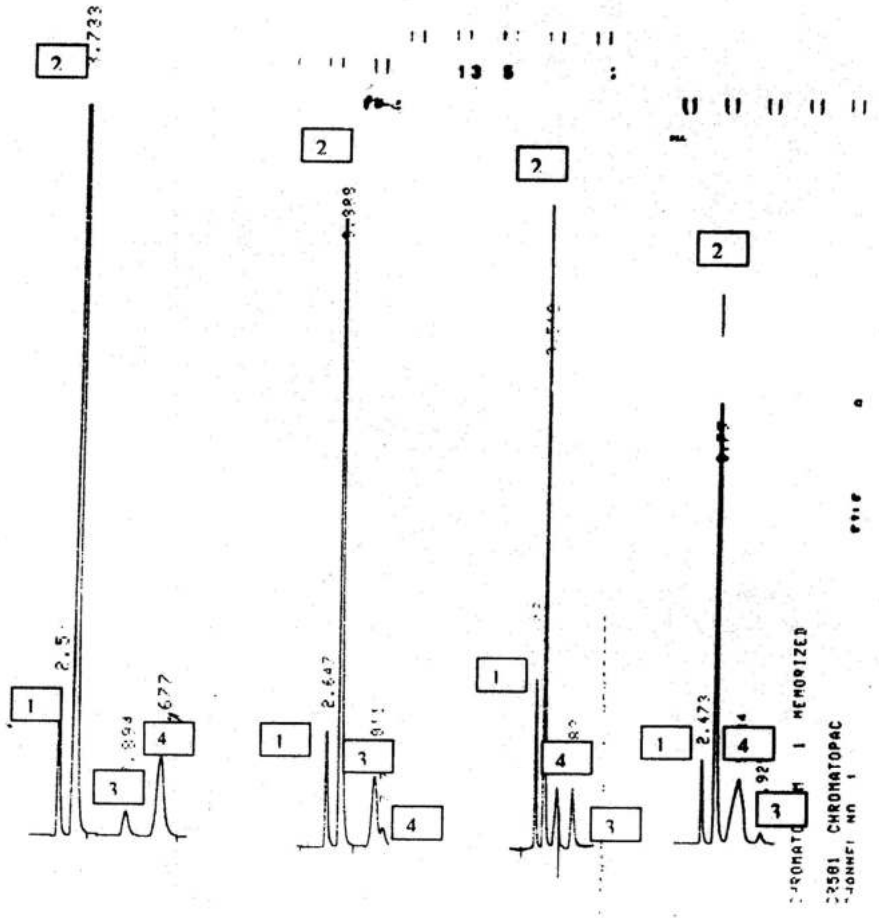


Fig. 1 Chromatogram showing variation in retention time of benzoic acid with change in pH

Table 1: Retention times of ascorbic acid, paracetamol, caffeine and benzoic acid at different pH

pH	MEAN RETENTION TIME/MIN			
	Ascorbic Acid	Paracetamol	Caffeine	Benzoic Acid (Internal Standard)
20	2.4982	3.6884	7.9494	10.7132
30	2.4564	3.6140	7.3146	9.465
40	2.4846	3.6612	7.6166	6.856
4.25	2.4236	3.6152	7.4452	5.3272
4.50	2.4538	3.6106	7.9762	4.762

Table 2: Limits of detection of ascorbic acid, paracetamol and caffeine using designed method

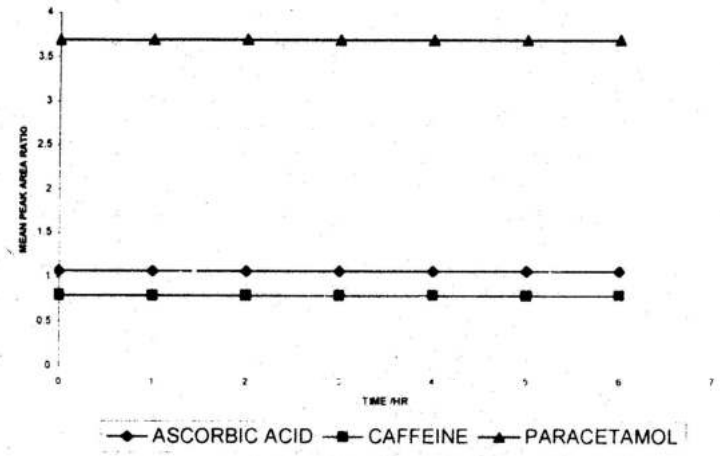
ASCORBIC ACID			PARACETAMOL			CAFFEINE		
Conc (µg/ml)	Relative Standard Deviation (%)	Signal/ Noise Ration	Conc (µg/ml)	Relative Standard Deviation (%)	Signal/ Noise Ration	Conc (µg/ml)	Relative Standard Deviation (%)	Signal/ Noise Ration
500	2.27	44.01	5000	1.22	81.71	300	4.06	
50	8.40	11.90	500	3.71	27.02	30	11.70	
5	11.59	8.62	50	6.84	14.61	3	24.27	24.6
0.5	27.01	3.70	5	23.25	4.30	0.3	-	8.53
0.05	-	-	0.5	-	-	0.03	-	4.12
								-
								-

Table 3: Calculated t values for the components at 95% confidence interval: $T_{CRICITAL} = 2.23$

Sample Code	Ascorbic Acid	Paracetamol	Caffeine
A1	1.299	0.8208	
A2	1.237	0.1146	
A3	1.258	0.8874	
A4	1.234	0.1063	

Table 4: Calculated f values for the components at 95% confidence interval: $F_{CRICITAL} = 5.05$

Sample Code	Ascorbic Acid	Paracetamol	Caffeine
A1	2.263	2.922	4.692
A2	1.974	2.782	3.005
A3	1.413	1.998	4.719
A4	3.819	1.689	2.637



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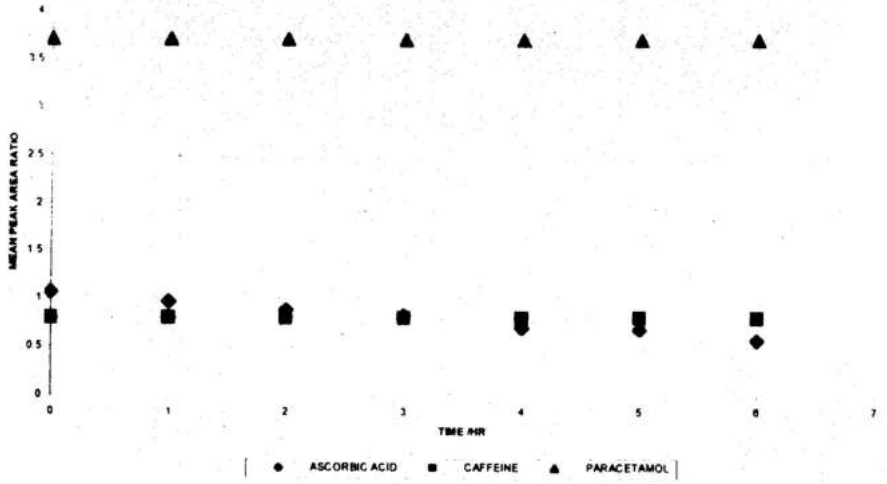
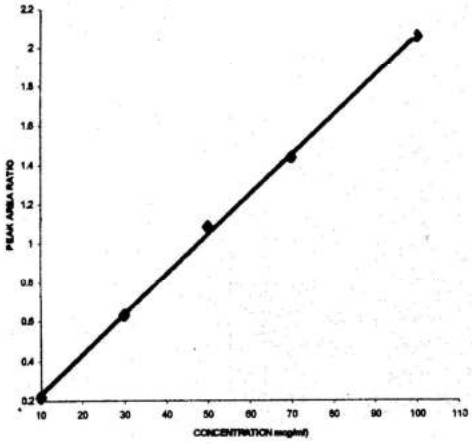
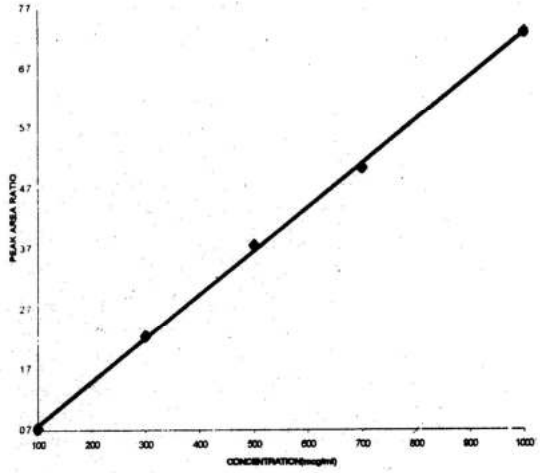
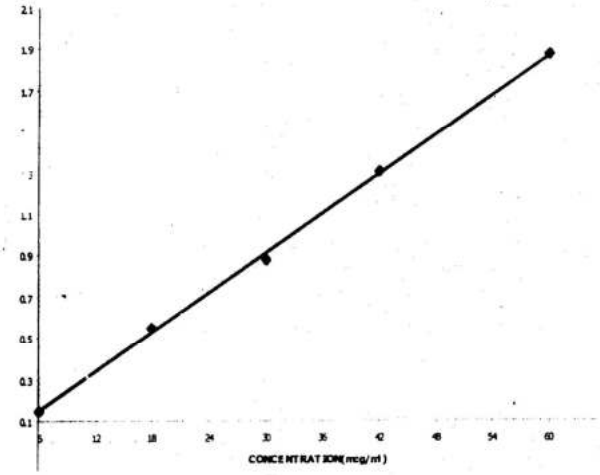


Fig. 2 Stability of Ascorbic acid, Paracetamol and Caffeine in (1) 0.04%$^{w}/_{v}$ $Na_2S_2O_3$ and (2) distilled water within day.


PEAK AREA RATIO
CONCENTRATION mcg/ml


PEAK AREA RATIO
CONCENTRATION(mcg/ml)


CONCENTRATION(mcg/ml)

RESULTS AND DISCUSSION

Design of Methods: A method for analysis of a combination of paracetamol and Caffeine using methanol: 0.05M KH_2PO_4 as mobile phase and C_{18} bonded stationary phase has been previously reported [Fisons, 1993]. An attempt was made to design a method based on the use of a C_8 stationary phase and mobile based on the above. The selection of methanol: phosphate buffer was based on the fact that buffered mobile phase, resist changes in pH so that the analyte will be consistently ionized to maintain consistent retention and selectivity resulting in reproducible chromatography. The C_8 column was used because fully reacted monomers of bonded C-8 packing represent a good compromise for reverse phase separations. These materials have moderate retention, good efficiency and stability, and a useful k' range for a wide variety of samples [Willard et al.,1988]. It was observed from early experiments that methanol: 0.05M NaH_2PO_4 (pH2) in the ratio 17:83, flow rate of 1.5ml/min, wavelength of detection 273nm and 0.3AUFS gave effective resolution of ascorbic acid, paracetamol and caffeine. Different compounds with pKa or structural similarity to the three compounds under investigation were selected and introduced unto the column. It was observed that benzoic acid gave retention time different from the others under the above chromatographic conditions and could act as internal standard. Total chromatographic time was found to be 11 minutes. The peak for benzoic acid was not symmetrical as tailing was observed (Fig.1). To establish effective balance between mobile and stationary phase and eliminate tailing alteration of mobile phase pH was thought of. Theoretically a mobile phase pH less than 1.5 the pKa of an analyte (benzoic acid pKa 4.2) [Clark, 1980] would decrease ionization and enhance solubility in the mobile phase thus eliminating tailing and decreasing retention time. Mobile phase pH was changed to 3. Interestingly this did not only enhance symmetry but decreased chromatographic time to 8.5 minutes. At pH 4 there was incomplete resolution between caffeine and benzoic acid, with benzoic acid eluting first (Fig.1, Table 1). This suggested that there was a fine pH at which benzoic acid would have a retention time between that of paracetamol and caffeine. This pH was found to be 4.25 ± 0.05. Thus this pH was used for analysis and total chromatographic time was 7.5 minutes. The detection wavelength of 273nm, 0.3AUFS ensured that the peak area ratios of t..e three drugs to the internal standard were close to unity.

Stability of ascorbic acid in 0.04%$^w/_v$ $Na_2S_2O_5$ has been reported [Osei-Asante, 1998]. This was selected as solvent for sample dissolution. Stability test were carried out to determine the stability of all other components in the solvent in the presence of benzoic acid within day and daily over a period of five days (Fig. 2). It was observed that all components were stable throughout the five-day period making this method extremely flexible. Mean retention times of ascorbic acid, paracetamol, benzoic acid and caffeine were 2.4, 3.6, 5.3 and 7.5 minutes, respectively (Table 1).

Analyte actual concentration was obtained from calibration curves based on the relationship between mean peak area ratio and the concentration (Fig. 3). The linearity between the detector response at 273nm and the analyte concentration was established for the three drugs. Linearity was obtained between 10-100µg/ml for ascorbic acid, 100-1000µg/ml for paracetamol and 6-60µg/ml for caffeine. Nominal anylate concentration was 50µg/ml of ascorbic acid, 500µg/ml of paracetamol and 30µg/ml and caffeine, respectively.

The regression equations (n=5) for the mean peak area ratio (y0 and concentration of drug (x, expressed in µg/ml) were $y = 203.18x + 0.0157$ for ascorbic acid, $y = 72.35x + 0.0479$ for paracetamol and $y = 318.06 - 0.0434$ for caffeine. Correlation coefficient was 0.9999 for all three curves suggesting a high level of linearity. The limit of sensitivity for this analysis 0.5µg/ml for ascorbic acid, 5µg/ml for paracetamol and 3µg/ml for caffeine. This suggested that the method could be used for detection of traces of these materials in samples where they appear as impurity.

Statistical Comparison of Designed Methods with Standard UV Method: The designed method and standard UV methods were used to analyze multi-component preparation on the market. The results obtained were compared statistically. The student t-test indicated that there was no significant difference between the means obtained from the two methods (Table3). It was observed that the standard, the relative standard deviation and standard error of the mean obtained for ascorbic acid, paracetamol and caffeine using the UV were higher than those from HPLC. This indicates that the HPLC had a lower margin of error compared with the UV method. The F-test showed

that there was no significant difference between the two methods in terms of precision and accuracy (Table 4).

Conclusion

An HPLC method was developed. Separation was achieved on a C_8 column with methanol: phosphate buffer (17:83) apparent pH 4.25 as mobile phase. Detection was at 273nm (0.3AUFS) using 0.04% $Na_2S_2O_3$ solution as the solvent for tablet dissolution. The experiment was done at ambient temperature. Ascorbic acid was found to be stable under the chromatographic conditions for five days. The HPLC method was found to be simple, rapid, selective, accurate, reproducible, and less time consuming compared to the UV method. It has the advantage of being used for single component products containing any of the mentioned active ingredients. This method also eliminated the extraction process, which is time consuming and associated with high error. It is also recommended that pharmaceutical companies and drug regulatory authorities adapt this method for analysis of products that contain these active ingredients.

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