Arch. Pharm. Res. **13**(3), 215-220

First Derivative Spectrophotometric and Gas-Liquid Chromatographic Determination of Caffeine in Foods and Pharmaceuticals III.

Simultaneous assay of caffeine and some antihistaminics

Ezzat M. Abdel-Moety§, Mohamed F. El-Tarras, Badr-Eldin A. El-Zeany and Khadiga O. Kelani

Department of Analytical Chemistry, Faculty of Pharmacy, Cairo University, Kasr El-Aini, ET-11562 Cairo, Egypt.

(Received January 8, 1990)

Abstract□Two different, derivative spectrophotometric and gas-liquid chromatographic, procedures for direct quantitation of caffeine and some commonly dispensed antihistaminics in bulk forms, in their laboratory prepared mixtures and in dosage formulations, have been investigated. The limit, sensitivity, reproducibility and accuracy of each method were studied for each individual drug substance and in some usual pharmaceutical formulations.

Keywords□Caffeine, antihistamine, first derivative, spectrophotometric determination, gas-liquid chromatographic determination.

Antihistaminic drugs are commonly described for the treatment of anaphylactic reactions, urticaria, rhinitis, pruritis and allergic conjunctivitis. They are also used for the prevention and treatment of nausea and vomiting, cough, motion sickness, sedation and hypnosis, which are urelated to their antihistaminic action[1]. The hypnotic characteristic of most of the well known and usually prescribed antihistamines is sometimes unfavoured due to caused loss of personal reflexes. An analyptic agent, mostly caffeine, is usually added to antagonise and rebalance such side effect of the antihistamines.

Several different analytical procedures have been described for determination of the antihistaminic drugs in pure and dosage forms[2-8] and other methods were recommended for quantitation of caffeine in bulk form, in foods and pharmaceutical preparations[4-8, 10].

The main task of the present work was to establish simple direct procedures for accurate determination of caffeine and some antihistamines simultaneously in mixtures and in dosage formulations. A derivative spectrophotometric method and another GLC procedure have been worked out for such an aim. Applicability of the proposed methods was studied for mixtures of caffeine with the maleate salts of mepyramine, pheniramine and chlorpheniramine, with or without phenylproponalamine hydrochloride, in some common medicinals.

EXPERIMENTAL METHODS

Apparatus

1. A Beckman DU-7 UV/visible videorecording spectrophotometer with matched 1 cm quartz cells, Beckman Instrum. Inc., California-USA.

2. A Pye-series 104, model 64, gas chromatograph with dual FID was attached to a Unicam AR25 linear recorder, Pye-Unicam, Cambridge-UK.

Authentic samples

Anhydrous caffeine (with labeled purity of 99.5%) and phenylpropanolamine HCl (99.6%) were donated from Misr Co. Pharm. Chem. Ind., El-Mataria, Cairo-ET. Pheniramine maleate (99.7%) was received from Hoechst-Orient SAE, Al-Amiria, Cairo-ET, while chlorpheniramine maleate (99.9%) was supplied by Kahira Pharm. Chem. Ind., Shoubra, Cairo-ET. Only caffeine was dried at 80°C for about 4 hrs. and cooled in a desiccator, while other samples were used without further treatment.

Pharmaceutical formulations

Tablets A: Each tablet contains (2 mg) chlorpheniramine maleate and (20 mg) caffeine, manufactured by Memphis Chem. Co., El-Zeiton, Cairo-ET.

Tablets B: Each tablet contains (25 mg) pheniramine maleate, (25 mg) mepyramine maleate, (50 mg)

§ Author for correspondence.

phenylpropanolamine HCl and (8 mg) caffeine, manufactured by Swiss-Pharma (Ciba-Geigy, Wander & Sandoz) Al-Amiria, Cairo-ET.

Analytical techniques

1. Spectrophotometric method

Calibration curves: Prepare separately standard series of each antihistamine and caffeine by dilution of prepared stock solutions, Ca. 10 mg·ml^{-1} in ethanol, using the same solvent. Measure the first derivative absorbance (d'A) of each concentration of chlorpheniramine or pheniramine maleate at about 273 nm [d'A (1%, 1 cm) = 12.76], mepyramine maleate at about 328 nm [(d'A (1%, 1 cm) = 25.00], and caffeine at about 289 nm [(d'A (1%, 1 cm) = 29.83)]. Plot the measured d'-absorbances at the determined λ_{max} against each corresponding concentration of individual compound to obtain calibration graphys.

Application of the derivative spectrophotometry to analyse dosage forms: Weigh accurately not less than twenty tablets, determine the average weight of each tablet and grind well in a glass mortar. Take from the homogenously mixed powdered tablets an accurate weight equivalent to about 25-50 mg of antihistamine. Dissolve in ethanol (Uvasol from E. Merck, Darmstad-FRG) by applying gentle heating, mechanical shaking can be applied to affect complete drug extraction, cool and complete to volume to get concentrations of about 4-20μg·ml^{-1}(ppm) of each component drug substance. Mix the contents of the ethanolic extract well, centrifuge an aliquot of the extract and measure the d'absorbances at the specified λ_{max} of each component drug and refer to the prepared calibration graphs. The d'A (1%, 1 cm)-values of the mentioned antihistaminics or caffeine can be taken as merit for direct calculation of the drug concentration in dosage forms.

2. Gas-liquid chromatographic procedure

Calibration curves: Prepare standard diluted series of each antihistamine or of caffeine to contain concentrations of 0.5-4 mg·ml^{-1} using absolute ethanol (E. Merck, Darmstadt-FRG). Inject 8-10μl amounts of each concentration in triplicates onto the GC-port under the following optimal operating conditions. Column: Glass column (150 cm × 6 mm id) packed with 3% methyl silicone gum (E-30) on 60-70 mesh diatomite C-AW DMCS, the column and support material were pre-silanized with dimethyl dichlorosilane (Pye-Unicam, Cambridge-UK). Isothermal operation at 220°C, detection and injection at 300°C. Carrier gas, N_2 (3 kPa/cm^2, flow rate = 44 ml·min^{-1}), other gases were air and hydrogen at 2 kPa/cm ^{2}). Chart speed 2 min·cm^{-1} at a normal sensitivity of 10 mV, attenuated at 30 × 10^2 and baking off range 1x. To construct the calibration curves, plot the peak area and the peak height against each concentration. To determine the concentration of a given drug substance in tablet extracts refer either to the prepared calibration curve or by comparing the obtained peak areas or heights of both unknown samples with that of a standard sample alternatively injected under the same condition. Barbitone, 5,5-diethyl barbituric acid (BDH Chemicals, Poole-UK) could be used as a good internal standard for correction and even for calculation of concentrations of component drug substances.

RESULTS AND DISCUSSION

The present work describes two different methods for simultaneous quantitation of the analeptic agent caffeine mixed or dispensed with some common antihistamines. The first method is based on applying the derivative spectrophotometry in order to solve the problem of band overlapping in analysing such multicomponent mixtures. In the other method an attempt has been made to detect the most appropriate GC-column for separation and quantitation of underivatized salts of antihistamines admixed with caffeine in laboratory prepared mixtures and in dosage formulations. A column packed with 3% methyl silicone gum (E-30) on diatomite C-AW DMCS (60-70 mesh) proved to be the most efficient column. There is no need to preliminary drug separation or to liberate the free base of the drug or sample clean-up as unnecessity to prepare a derivative.

Derivative spectrophotometry

UV-scanning of ethanolic solutions of mixtures containing caffeine and chlorpheniramine maleate show band overlap of the maxima of caffeine at 273 nm and or chlorpheniramine maleate at 262 nm in the zero-order (D_0), Fig. 1a. On the other hand, the first-derivative (D_1) scanning of the same binary mixtures of the individual drug substances exhibits that caffeine has a typical trough at about 288 nm while chlorpheniramine maleate shows zero or negligible absorption reading at that wavelength. Similarly, when chlorpheniramine maleate reads maximally at about 273 nm, caffeine has no measurable absorbance, Fig. 1b. On the bases of such observations, it would be possible then to adopt derivative spectrophotometry for the determination of both drug substances simulataneously in their mixtures. Linear relationship between the amplitude height in the D_1-spectra [d'A/d λ , i.e. (d'A)] and the concentration of caffeine and chlor-

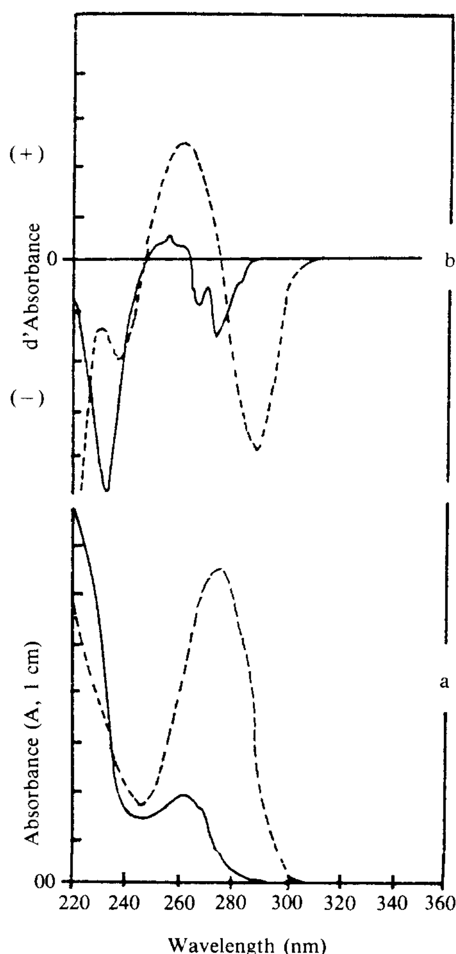

Fig. 1. The UV-scanning [(a) = zero-order; D_0 and (b) = 1st. derivative; D_1] of ethanolic solutions of chlorpheniramine maleate (—) and caffeine (--).

pheniramine maleate have been found in the ranges of 8-40μg·ml^{-1}(ppm), respectively. Statistical evaluation of the obtained results demonstrates low figures of relative standard deviation (SDr) for caffeine and chlorpheniramine maleate (0.006 and 0.007, respectively) on applying the D_1-spectrophotometric technique. Standard additions of authentic caffeine and chlorpheniramine maleate to powdered tablets showed excellent mean recoveries with low deviations, 99.26±0.82% (n = 5) and 99.24±0.87% (n = 5), respectively. This indicates the high accuracy and precision of the adopted derivative spectrophotometric method. The described procedure could be also adopted for simultaneous quantitation of caffeine admixed or dispended with pheniramine maleate, mepyramine maleate with or without phenylpropanolamine hydrochloride. The D_1-curves of caffeine and the maleate salts of pheniramine and mepyramine show that caffeine has its typical trough at about 288 nm while the antihistamincis read almost zero. On the other hand, pheniramine maleate shows maximal d'absorption at 273 nm whereas both caffeine and mepyramine maleate have zero-readings. Similarly, mepyramine maleate exhibits its maximal d'absorption at 238 nm where other components give zero or negligible responses. Through the specified peaks, it would be possible then to quantify the studied drug substances singly or in mixtures. The response of phenylpropanolamine hydrochloride, which is sometimes dispensed with the studied analeptic and/or antihistaminic drugs exhibits no D_1-absorptions at 273 nm, 288 nm or at 328 nm. So, its presence together with such drug substances would not interfere in their quantification by adopting the D_1-spectrophotometry. Fig. 2a & b shows the D_0 and D_1-scanning, respectively, of ethanolic solution of caffeine, mepyramine and pheniramine maleates.

Ethanolic solutions of caffeine or pheniramine maleate in the range of 4-40μg·ml^{-1} and mepyramine maleate in the range of 1-16μg·ml^{-1} showed significant linearity. The relative standard deviations were less than 0.01 on adopting the D_1-spectrophotometry for caffeine (0.055), pheniramine maleate (0.009) and mepyramine maleate (0.009). Slope constancy of prepared calibration graphs of each individual drug substance singly or in mixtures at its specified λ_{max} showed excellent reproducibility. Recovery percent of added reference authentic caffeine, pheniramine maleate and mepyramine maleate to labeled amounts in dosage formulations were excellent, [99.04±0.82 (n = 8)], [99.09±1.23 (n = 8)] and [100.35±1.04 (n = 8)], in order.

Gas-liquid chromatography (GLC)

In an attempt to detect the most appropriate GC-column for separation of the underivatized salt of antihistamine, several different columns, namely 10% dinonyl phthalate (DNP) on diatomite C-AW, 5% xylenyl phosphate (XPH) on gas-chrom. Q or diatomite C-AW, 10% squalane on celite and gas-chrom. Q; 5% & 10% silicone oil on diatomite C-AW, and 3% methyl silicone gum (E-30) on diatomite C-AW, were tried. The last one, i.e. 3% E-30 on diatomite C-AW DMCS (60-70 mesh) proved to be the most efficient column. There is no need to preliminary drug separation or sample clean-up as well as unnecessity to prepare a derivative or to liberate the free base of the drug (except in case if there is colouring matter and or sugar are added) where all these save time. The method is rapid, so a complete assay of pure drugs,

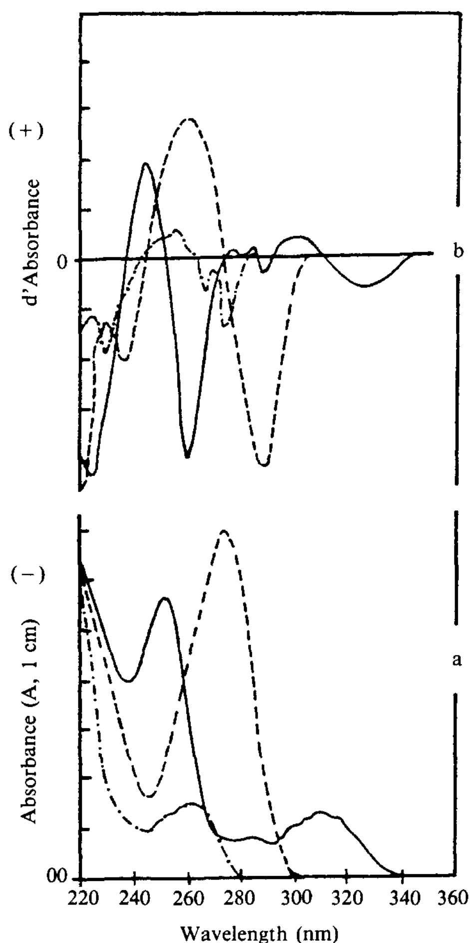

Fig. 2. The UV-scanning [(a) = zero-order; D_0 and (b) = 1st derivative; D_1] of ethanolic solutions of mepyramine maleate (—), pheniramine maleate (- · -) and caffeine (--).

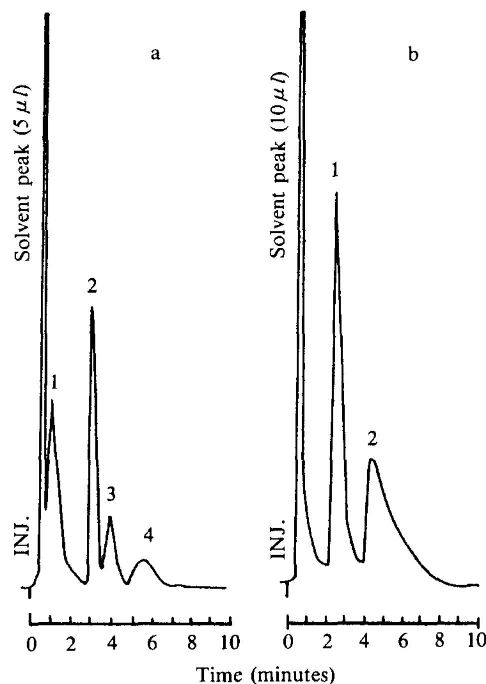

Fig. 3 Typical chromatographic resolution of (a) = phenyl propanolamine HCl (1), caffeine (2), pheniramine maleate (3) and mepyramine maleate (4); and (b); caffeine (1) and chlorpheniramine maleate (2).

as salts, needs about 5 minutes and a sample of any-one of the analyzed pharmaceutical preparations needs not more than 15-20 minutes, the R_t values are about 1.6-3.8 minutes. Satisfactory peaks were obtained, by maintaining the column isothermally at the specified temperature for each compound, where a linear relationship could be obtained at a concentration ranges of 0.5-4 $mg \cdot ml^{-1}$ of the drug in ethanol. It was observed that the results calculated from the peak-area are more accurate than those calculated from the peak-height. The suitability of the calibration curves for quantitative analysis was checked by adding barbitone as an internal standard; the results obtained were more or less the same as those obtained from refering to the calibration curve.

Table I, shows the results obtained from the described GLC-method for determination of single antihistamine. On subjecting these results to statistical comparison with the results obtained by the official methods, it was found that the calculated values of both student's-t and F at ($p = 0.05$) are less than the tabulated values. This reveals that there is no significant differences between the accuracy and the precision of both methods. Moreover, the GLC-method is advantageous over the official method in that less quantity of antihistamine is required. In addition, the official methods are not selective to differentiate between those drugs when present in mixtures. The antihistamines are commonly formulated together with other compounds, such as caffeine and phenylpropanolamine hydrochloride, where there is no pharmacopoeial methods are recommended for the simultaneous anlysis of such mixtures without preliminary chromatographic separation or derivatization. Chlorpheniramine-caffeine mixtures were GLC-analyzed, first in laboratory prepared mixtures with an accuracies of $100.32 \pm 0.83\%$, for chloropheniramine maleate, and $100.92 \pm 0.84\%$, for caffeine. The mean percentage recoveries of separately added chlor-

Table I. Analyses of chlorpheniramine maleate, pheniramine maleate, mepyramine maleate and caffeine by the proposed derivative spectrophotometric and gas-liquid chromatographic procedures with official methods

	Chlorpheniramine maleate		Caffeine	
	D_1, (%)	GLC, (%)	D_1, (%)	GLC, (%)
Authentic	100.01 ± 0.48 (n = 10)	99.47 ± 0.73 (n = 7)	99.92 ± 0.57 (n = 9)	99.91 ± 0.82 (n = 6)
Student's-t	0.164* (2.262)**	0.244* (2.447)	0.233° (2.306)	0.234° (2.571)
Binary mixtures	99.09 ± 0.85 (n = 12)	100.32 ± 0.83 (n = 6)	100.02 ± 0.83 (n = 12)	100.92 ± 0.84 (n = 6)
	Pheniramine maleate		Mepyramine maleate	
Authentic	100.01 ± 0.48 (n = 10)	100.42 ± 0.65 (n = 6)	99.92 ± 0.64 (n = 8)	99.43 ± 0.66 (n = 6)
Student's-t	0.103* (2.262)	1.932* (2.571)	2.170° (2.365)	1.193° (2.571)
Binary mixtures	99.09 ± 1.23 (n = 8)	99.77 ± 0.60 (n = 8)	100.35 ± 1.04 (n = 8)	100.32 ± 0.80 (n = 8)

*The results compared with those of BP 1988[11].
**Figures in parentheses are the corresponding tabulated t-values at n-1 degree of freedom and 0.05 degree of probability.
°The results compared with those of USP 1986[12].

Table II. Assay and recovery of added antihistamines and caffeine in pharmaceutical formulations by applying the proposed spectrophotometric and gas-liquid chromatographic procedures

Pharmaceutical formulation*	d'Spectrophotometry			GLC-procedure		
	Chlorpheiramine maleate	Caffeine		Chlorpheniramine maleate		Caffeine
Tablet A						
Assay** (%) =	99.04	99.39		98.91		100.12
Recovery % (X ± SD) =	99.24 ± 0.87 (n = 5)	99.26 ± 0.82 (n = 5)		99.84 ± 0.67 (n = 10)		99.84 ± 0.82 (n = 10)
	Pheniramine maleate	Mepyramine maleate	Caffeine	Mepyramine maleate	Mepyramine maleate	Caffeine
Tablet B						
Assay** (%) =	99.95	101.17	97.28	99.95	102.09	101.07
Recovery % % (X ± SD) =	99.09 ± 1.23 (n = 8)	100.35 ± 1.04 (n = 8)	99.04 ± 0.82 (n = 7)	99.97 ± 0.60 (n = 8)	100.32 ± 0.80 (n = 8)	100.03 ± 0.78 (n = 8)

*More details about claimed composition, see Experimental Section.
**Mean of at least five experiments carried out separately.
°Contents of phenylproponolamine HCl was assessed by the proposed GLC-procedure and found to be 50.08 mg/tablet (100.15%).

pheniramine maleate and caffeine to powdered tablets A were 99.84 ± 0.67%, and 99.84 ± 0.52%, respectively.

Prepared mixtures of pheniramine maleate, mepyramine maleate, phenylpropanolamine hydrochloride and caffeine were simultaneously determined by the proposed GLC-method, the corresponding accuracies were 100.02 ± 0.61%, 100.28 ± 0.69%, 99.71 ± 0.74% and 99.77 ± 0.62%, respectively. The mean percentage recoveries of added pheniramine maleate, mepyramine maleate, phenylproponolamine hydrochloride and caffeine separately to the tablets B were found to be 99.77 ± 0.60%, 100.32 ± 0.80%, 100.74 ± 0.83%, and 100.03 ± 0.80%, in order, Table II.

The proposed methods have been applied for simulataneous quantification of caffeine in admixtures with procaine · HCl (Novocaine) in geriatrics[13] and determination of the analeptic drug in coffee, tea and soft drinks[14].

It can be concluded that the proposed GLC-method is simple, and also rapid, accurate and precise for the determination of antihistamines either singly or in mixtures, with or wihtout added caffeine.

ACKNOWLEDGEMENT

The authors would like to thank the pharmaceutical companies, Misr Co. Pharm. Chem. Ind., Hoechst-Orient SAE and Kahira Pharm. Chem. Ind., in Cairo-ET, for kind donation of authentic drug substances.

LITERATURE CITED

1. Burger, A. (Ed.): Medicinal Chemistry, 2nd Edn., Inc., New York, p.531, (1960).
2. Ismaiel, S.A.: Determination of antazoline and diphenhydramine in some pharmaceutical preparations containing ephedrine, *Pharmazie,* **29,** 423 (1974).
3. Matsui, F. and French, W.N.: Analysis of binary mixtures of pharmaceutical amines by the acid-Dye technique, *J. Pharm. Sci.* **60,** 287 (1971).
4. Sakai, T. and Ohno, N.: Use of thermochromism of associate for the determination of antihistamine drugs in pharmaceutical preparations and urine, *Analyst,* **107,** 634 (1982).
5. Sperling, A.R.: Analysis of phenothiazine · HCl in syrups, *J. Pharm. Sci.* **56,** 98 (1967).
6. Cosofert, V.V. and Buck, R.P.P.: Phenothiazine drugs poly (vinyl chloride) matrix membrane electrode and their use in pharmaceutical analysis, *Analyst,* **109,** 1321 (1984).
7. Newton, J.M.: Spectrophotometric determination of caffeine in coffee products (Collaborative Study), *J. Assoc. Off. Anal. Chem.* **62,** 705 (1979).
8. Sjoberg, A.M. and Rajama, J.: Simple method for the determination of alkaloids in cocoa using paper chromatography and UV-spectrometry, *J. Chromatogr.* **295,** 291 (1984).
9. Vitzthum, O., Barthels, M. and Kwasny, H.: Rapid method for the determination of caffeine in caffeine-containing and decaffeinated coffee by gas chromatography with the nitrogen-sensitive detector, *Z. Lebensm. Unters.-Forsch.* **154,** 135 (1974).
10. Blauch, J.L. and Tarka Jr., S.M.: HPLC-Determination of caffeine and theobromine in coffee, tea and instant hot coca mixes, *J. Food Sci.* **48,** 745 (1983).
11. The British Pharmacopoeia 1988, HMS Office, London, pp.84,131,359, & 428.
12. The United States Pharmacopoeia XXI, US Pharmacopoeial Convention, Rockville-MD., 1985, pp.143,202, & 921.
13. Abdel-Moety, E.M. and Moustafa, A.A.: First-derivative spectrophotometric and gas-liquid chromatographic determination of caffeine in food products and pharmaceuticals. II. Simultaneous assay of caffeine and procaine hydrochloride (Novocaine) in binary mixtures and in geriatrics, *Norv. Pharm. Acta,* **48,** 75 (1986).
14. Abdel-Moety, E.M.: First-derivative spectrophotometric and gas-liquid chromatographic determination of caffeine in foods and pharmaceuticals. I. Rapid determination of caffeine in coffee, tea and soft drinks, *Z. Lebensm. -Unters. Forsch.* **186,** 412 (1988).