ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
30 (2002) 859–867

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Short communication

Determination of some histamine H_1-receptor antagonists in dosage forms

Azza A. Gazy, Hoda Mahgoub, F.A. El-Yazbi *, M.A. El-Sayed, Rasha M. Youssef

Department of Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, University of Alexandria, Alexandria 21521, Egypt

Received 17 January 2002; received in revised form 3 May 2002; accepted 9 May 2002

Abstract

Three simple and accurate methods are presented for determination of Cetirizine, Fexofenadine, Loratadine and Acrivastine in pure form and commercial dosage forms. The first method is based on the reaction of the above cited drugs with bromocresol purple dye to form ion-pair complex extractable with chloroform and subsequently measured spectrophotometrically. Secondly, eosin gives with these drugs ion-pair complex, measurable directly without extraction both spectrophotometrically and spectrofluorimetrically. The last method involves the base-catalysed condensation of mixed anhydrides of organic acids (citric acid/acetic anhydride) where as the tertiary amino group in the above-cited drugs acts as the basic catalyst. The product of condensation is measured spectrophotometrically. All the reaction conditions for the proposed methods have been studied.

Keywords: Histamine antagonist; Eosin; Spectrophotometrically; Spectrofluorimetrically; Citric acid

1. Introduction

Cetirizine (CET), Fexofenadine (FEX), Loratadine (LOR) and Acrivastine (ACR) are potent, long-acting histamine H_1-receptor antagonist [1].

Many procedures are described for quantitative determination of CET, FEX, LOR and ACR. Among these methods are high performance liquid chromatography [2–5], gas chromatography [6–8], polarography [9,10] and spectrophotometry [11,12].

Colorimetric and fluorimetric methods were reported for the analysis of LOR [13,14], CET [15,16] and ACR [17,18]. No spectrophotometric or colorimetirc methods were reported for the determination of FEX.

LOR, ACR and FEX are not compendial drugs while CET was officially assayed potentiometrically [19].

* Corresponding author. Tel.: +20-3-4871317; fax: +20-3-4873273

E-mail address: pharmacy_alex@hotmail.com (F.A. El-Yazbi).

0731-7085/02/$ - see front matter
PII: S0731-7085(02)00318-7

Compounds have basic cationic nitrogen react with anionic dye at a suitable pH, to form highly colored ion pair complex [20,21]. Also tertiary amines has been determined through base-catalyzed condensation of mixed anhydride of organic acids, where colored products are formed [22,23].

In this paper, CET, FEX, LOR and ACR are determined through formation of ion pair complex with bromocresol-purple (BCP) and eosin, as acidic dyes. The presence of tertiary amino group in the above-cited drugs acts as the basic catalyst for the condensation reaction of citric acid/acetic anhydride (CAA).

2. Experimental

2.1. Apparatus

1. Perkin-Elmer Lambda EZ 201 with Panasonic 24 Pin Quit KX 3626 printer.
2. Perkin-Elmer 650-10S fluorescence spectrophotometer with Perkin-Elmer Model 56 recorder.
3. Gerate pH meter Model CG 710 Calibrated with standard buffer at room temperature.

2.1.1. Materials and reagents

All materials used were of analytical reagent grade.

2.1.1.1. Material.

1. CET HCl: kindly supplied by Amriya Pharm. Company, Alexandria, Egypt.
2. FEX: kindly supplied by Hoechst, Marion, Roussel Alexandria, Egypt.
3. LOR micronized: kindly supplied by Pharaonia Pharmaceuticals, Alexandria, Egypt.
4. ACR: kindly supplied by Amriya Pharm. Company.

2.1.1.2. Reagents.

- BCP (Aldrich) 2×10^{-3} M in water, chloroform BDH, anhydrous sodium sulfate (Prolabo), methanol, ethanol Analar BDH, Acetonitril HPLC grade, Acetic anhydride (Prolabo) and eosin (Riedel-DE-Haen AG seeize—Hannover) 1.5×10^{-3} M in water were used.
- Mclivaine Buffer solutions (pH range from 2.2 to 8) were prepared by mixing specific volumes of disodium hydrogen phosphate (0.2 M) and citric acid (0.1 M).
- Methylcellulose solution (MC) Prolabo was prepared as 0.5% w/v in water with the aid of heat.
- CAA reagent is saturated solution of Citric acid (Merck) in acetic anhydride.

2.1.2. Standard solutions

Stock solution of CET, FEX, LOR and ACR containing 0.1 mg/ml were prepared in methanol.

2.2. Procedure

2.2.1. Construction of calibration curves for BCP method

Four series of 60 ml separating funnels, transfer different aliquots of standard solutions of CET, FEX, LOR and ACR within concentration range cited in Table 1. Add 5 ml of (2×10^{-3} M) BCP solution for CET, 2 ml for FEX, LOR and 3 ml for ACR, followed 1 ml of Mclivaine's citric acid phosphate buffer pH 2.6 for CET, pH 2.4 for FEX and ACR and pH 2.2 for LOR. The total volume of each solution was completed to 10 ml with water. The formed complex was extracted with 3×3 ml portions of chloroform, the solution was shaken for 1 min each time and the chloroform layer was passed through a layer of anhydrous sodium sulphate into 10-ml volumetric flask. The volume was made up to 10 ml with chloroform and the absorbance of the solution was measured at the corresponding λ_{max} Table 1 against a reagent blank.

2.2.2. Construction of calibration curves for eosin method

To four sets of 10-ml volumetric flasks, different aliquots of the standard solutions of CET, FEX, LOR and ACR within concentration range cited in Table 1, were pipetted each into its corresponding set, then 0.5 ml of (0.5 g%) MC (0.7 ml for LOR) was added, followed by 1.5 ml of eosin

Table 1
Statistical data of the regression equations for determination of (CET, FEX, LOR and ACR) with the proposed methods

Parameter	CET				FEX			
	BCP method	Eosin method	Fluorimetric method	CAA method	BCP method	Eosin method	Fluorimetric method	CAA method
Concentration range (μg/ml)	5–15	8–24	3–10	3–7	5–30	6–20	0.002–0.01	5–16
λ_{max} (nm)	407	539	550	543	412	541	545	543
λ_{ex} (nm)	–	–	310	–	–	–	310	–
ε	2.85×10^{4}	1.72×10^{4}	–	8.48×10^{4}	1.66×10^{4}	2.65×10^{4}	–	2.8×10^{4}
(a)	−0.00282	0.00219	−0.5131	−0.0167	−0.0047	0.03603	0.28	0.0103
(b)	0.06184	0.03737	2.559	0.184	0.03319	0.051139	3.990	0.056
(r)	0.999	0.9993	0.999	0.9997	0.9993	0.999	0.9994	0.9996
S_a	0.01277	0.01276	0.4050	0.01287	0.0125	0.0127	0.5292	0.00882
S_b	0.00132	0.000674	0.0645	0.00247	0.00068	0.000911	79.791	0.000766
S_b^2	1.74×10^{-6}	4.5×10^{-7}	4.16×10^{-3}	6.1×10^{-6}	4.74×10^{-7}	8.29×10^{-7}	6366.4	5.86×10^{-7}
Detection limit	0.284	1.3	–	0.29	0.68	0.58	–	0.475
Quantitation limit	0.692	4.5	–	0.75	1.9	3.59	–	2.01
	LOR				ACR			
	BCP method	Eosin method	Fluorimetric method	CAA method	BCP method	Eosin method	Fluorimetric method	CAA method
Concentration range (μg/ml)	20–55	3–10	0.004–0.01	5–16	6–20	3–15	2–10	3–9
λ_{max} (nm)	409	539	550	543	410	540	660	543
λ_{ex} (nm)	–	–	330	–	–	–	330	–
ε	0.64×10^{4}	5.05×10^{4}	–	2.24×10^{4}	1.78×10^{4}	3.98×10^{4}	–	3.57×10^{4}
(a)	0.00073	−0.0381	0.05948	2.6×10^{-3}	−0.0104	0.0093	−0.257	0.0122
(b)	0.0167	0.1285	3.1818	0.0587	0.05110	0.11429	3.0291	0.1026
(r)	0.9999	0.998	0.9998	0.9993	0.9996	0.9988	0.996	0.9996
S_a	0.00318	0.0228	0.21117	0.01334	0.008	0.0267	0.2723	0.00872
S_b	8.13×10^{-5}	0.0033	30.417	0.00156	0.0005	0.00279	0.0445	0.00135
S_b^2	0.661×10^{-5}	1.11×10^{-5}	925.19	2.43×10^{-6}	3.36×10^{-7}	7.78×10^{-7}	1.98×10^{-3}	1.822×10^{-6}
Detection limit	1.98	0.572	–	0.584	0.34	0.64	–	0.24
Quantitation limit	6.5	1.214	–	2.051	3.67	2.36	–	1.07

ε, Apparent absorptivity; (a), intercept; (b), slope; (r), correlation coefficient; S_a, standard deviation of Intercept; S_b, standard deviation of slope; S_b^2, variance around slope.

solution (2 ml for ACR) and 1 ml of Mclivaine's citric acid phosphate buffer (1.5 ml for CET) pH 3.0 (pH 2.6 for LOR and ACR). Each set was allowed to stand for 10 min at room temperature ($25 \pm 5°$) then diluted to volume with water. The absorbance was measured at the corresponding λ_{max} Table 1 against a reagent blank.

2.2.3. Construction of calibration curves for fluorimetric method

Into a set of 10-ml volumetric flask, separately transfer 1 ml of standard solution of each drug and complete procedure as under Section 2.2.2. Volumes of the above solutions within concentration range cited in Table 1 were pipetted either in 10-ml volumetric flasks (for CET, ACR) or in 100-ml volumetric flasks (for FEX, LOR) and completed to the volume with distilled water (ethanol for FEX and acetonitrile for LOR). The difference in the relative fluorescence intensities was measured at λ values cited in Table 1 against reagent blank.

2.2.4. Construction of calibration curves for CAA method

Into four sets of wide mouth thick wall test tubes, aliquots of the drugs within the concentration range cited in Table 1 were pipetted each into its corresponding set and evaporated to dryness in a hot water bath. Then 5 ml of CAA reagent was added, and each set was allowed to stand in a boiling water bath for 30 min. After cooling, the contents of each test tube was transferred quantitatively into 10-ml volumetric flask and the volume was completed with methanol. The intensity of the developed violet color was measured at 543 nm against a blank solution treated similarly.

2.2.5. Assay of drugs in pharmaceutical formulations

2.2.5.1. Tablets. Ten tablets of each of CET, FEX and LOR were accurately weighed and ground into a fine powder. A weight of powder equivalent to 10 mg of each drug was transferred into 100-ml volumetric flask, dissolved in water (for CET) and methanol (for FEX, LOR) then completed to volume with the same solvent. The flask was shaken for 30 min then filtered through Whatman No 41 filter paper.

2.2.5.2. Capsules. The contents of ten capsules were emptied and a known weight of the powder equivalent to 10 mg of ACR was transferred into 100-ml volumetric flask and extracted by shaking with 100 ml methanol for 30 min, then the contents were filtered.

2.2.5.3. Syrup. An aliquot volume of syrup equivalent to 5 mg of LOR was pipetted into 50-ml volumetric flask and completed to volume with methanol.

The procedures were completed on the filtrate of tablets and capsules and on the syrup solution as described in Section 2.2.1 for BCP method, Section 2.2.2 for eosin method, Section 2.2.3 for fluorimetric method and Section 2.2.4 for CAA method.

3. Results and discussion

3.1. BCP method

Containing cationic nitrogen, the cited drugs react with BCP to form colored ion-pairs extractable with chloroform and measured at 410 nm. Optimum reaction conditions including effect of buffer pH, buffer volume and BCP volume were clearly investigated in order to reach maximum sensitivity and low blank reading for the four cited drugs. It was found that 2 ml of BCP solution (5 ml for CET and 3 ml for ACR) and 1 ml of Mclivaine's citric acid phosphate buffer pH 2.6 for CET, pH 2.4 for FEX and ACR and pH 2.2 for LOR to gave maximum sensitivity (Figs. 1 and 2).

Some organic solvents e.g. chloroform, toluene, chlorobenzene and methylene chloride was applied for extraction of the complex. It was found that chloroform was the most ideal solvent that yielding maximum absorbance intensity and lowest blank reading. Shaking time for about 1 min produced reproducible absorbance. The developed color of the different extracts was stable for more than 1 h.

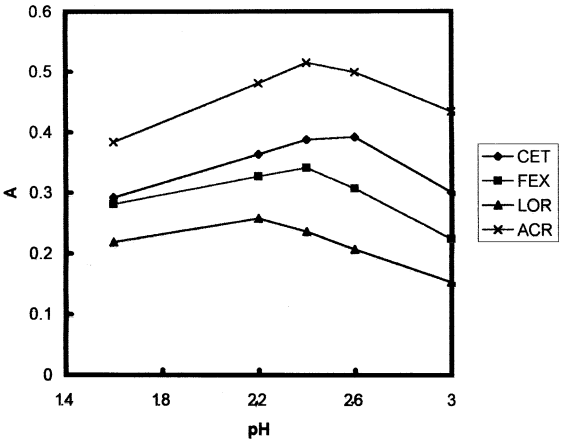

Fig. 1. Effect of pH on the chromogen formation between BCP and (6.5, 10, 20, and 10 μg/ml) of CET, FEX, LOR and ACR, respectively.

3.2. Eosin method

The four antihistaminic drugs CET, FEX, LOR and ACR reacted with eosin through an ion-pair salt formation, forming a reddish orange chromophore with λ_{max} at 540 nm. The ion-pair formation was optimized using 1.5 ml of eosin (2 ml for ACR). The maximum color was obtained when 1 ml of Mclivaine's citric acid phosphate buffer (1.5 ml for CET) pH 3.0 (pH 2.6 for LOR and ACR) (Fig. 3).

Various surfactants such as sodium lauryl sulphate, methyl cellulose, Tween 20 and Tween 80

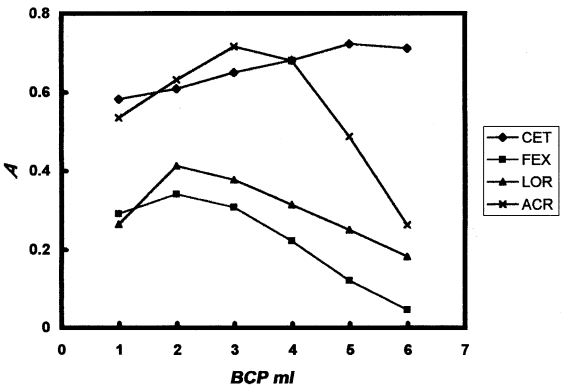

Fig. 2. Effect of BCP volume (2×10^{-3} M) on the chromogen formation with (12, 10, 25 and 15 μg/ml) of CET, FEX, LOR and ACR, respectively.

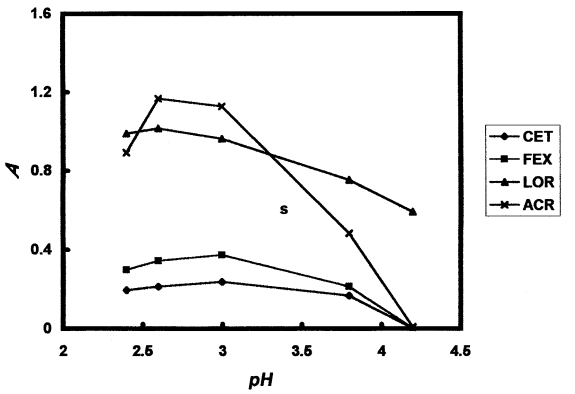

Fig. 3. Effect of pH on ion pair complex formation between eosin and (8, 7, 8 and 10 μg/ml) of CET, FEX, LOR and ACR, respectively.

were tried to increase the solubility of the formed complex and the highest sensitivity was obtained when 0.5 ml methyl cellulose solution was used (0.7 for LOR). The color developed using the above stated optimum conditions was found to be stable for at least 1 h.

3.3. Fluorimetric method

It was investigated that due to the ion-pair complex formation between the cited drugs and eosin, reduction of fluorescence may occur. The relative fluorescence intensity decreased significantly compared with the blank. The magnitude of the decrease was proportional to the concentration of the drug. The emission and excitation wavelengths were shown in Table 1.

Job's method [24] was applied in order to study the stoichiometry of the reaction of the four antihistaminic drugs and the dyes used (BCP and eosin). Results revealed that 1:1 complexation ratio under the optimum conditions were attained for the reaction between the four drugs and both dyes.

3.4. CAA method

Under suitable conditions, citric acid and acetic anhydride condensed with CET, FEX, LOR and ACR (containing a tertiary amino group) to give colored condensation products. The color formed

is possibly due to traces of alkalis on the glassware which catalyze the condensation reaction ([22]). CAA reagent gave a violet-colored product with maximum at about 543 nm.

Several experiments were conducted to fix optimum parameters, viz. reagent volume, temperature and time for the reaction. It was established that 5 ml of CAA was required for maximum color development (Fig. 4). Regarding the heating time and temperature, it was appears that 30 min in boiling water-bath give maximum sensitivity.

3.5. Validation of the methods

Using the above mentioned spectrophotometric and fluorimetric procedures, linear regression equations were obtained over the concentration ranges stated in Table 1. The statistical parameters, regression equations and S.D. of the slope (S_b) are given in Table 1. The good linearity of the calibration graphs is clearly evident from the values of the variances around the slopes (S_b^2) and the detection limits varied from 0.24 to 1.98 μg/ml.

In order to evaluate the precision of the proposed methods, solutions containing three different concentrations of the stated drugs were prepared and analysed in five replicates. The analytical results obtained from this investigation are summarized in Table 2. The low values of the relative standard deviation (R.S.D.%) and percentage relative error (Er%) also indicate the high precision and the good accuracy of the proposed methods Table 2.

The influence of commonly used tablet and capsule excipients (lactose, starch, magnesium stearate, talc and microcrystalline cellulose) and syrup diluent was investigated before the determination of the drug in dosage forms. No interference could be observed with the proposed methods.

3.6. Analysis of commercial dosage forms

The applicability of the proposed methods was tested by the determination of the four antihistaminic drugs in commercial dosage forms (tablets, capsules and syrup).

The determination was carried out on the same batch of samples together with reference method (A_{max}). The results obtained were presented in Table 3. The calculated t- and F-values did not exceed the theoretical values, indicating no significant difference between the methods.

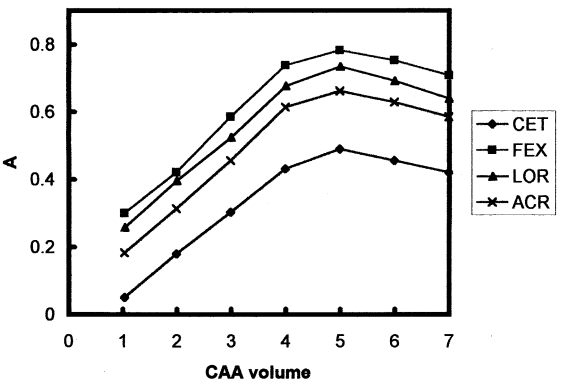

Fig. 4. Effect of reagent volume on the reaction of (3, 13, 12 and 6 μg/ml) of CET, FEX, LOR and ACR, respectively, with CAA reagent.

4. Conclusion

The proposed spectrophotometric and fluorimetric methods are suitable for the analysis of the four antihistaminic drugs in commercial dosage forms. The BCP and eosin methods are of equal sensitivity, however the eosin method has an advantage that it required no extraction procedure. The fluorimetric method is more selective and more sensitive than the spectrophotometric methods. The CAA method was shown to be simple, inexpensive, selective (for tertiary amino group), accurate and sensitive. The present methods are useful and convenient for quality control and routine determination of drugs in pharmaceutical dosage forms.

Table 2
Evaluation of the precision of the proposed methods for CET, FEX, LOR and ACR

Drug	BCP method			Eosin method			Fluorimetric method			CAA method		
	Added[a]	Rec. ± S.D.[b]	Er%[c]	Added	Rec. ± S.D.	Er%	Added	Rec. ± S.D.	Er%	Added	Rec. ± S.D.	Er%
CET	8	99.7 ± 1.08	−0.3	12	100.3 ± 1.41	0.3	5	99.8 ± 1.12	−0.2	3	101.9 ± 1.03	1.9
	10	100.4 ± 0.95	0.4	16	100.73 ± 0.82	0.73	7	99.8 ± 0.76	−0.2	5	101.08 ± 0.98	1.08
	15	98.5 ± 1.13	−1.5	22	101.08 ± 1.63	1.08	9	100.6 ± 1.36	0.6	7	100.3 ± 1.32	0.3
FEX	10	100.6 ± 0.54	0.6	8	98.9 ± 1.52	−1.1	0.002	100.7 ± 0.57	0.3	5	99.6 ± 2.0	−0.4
	16	99.2 ± 1.54	−0.8	17	98.92 ± 1.43	−1.08	0.006	100.3 ± 1.37	0.3	10	99.3 ± 0.75	−0.7
	30	100.1 ± 0.7	0.1	20	99.74 ± 0.54	−0.26	0.01	100.6 ± 1.14	0.6	15	100.5 ± 0.8	0.5
LOR	25	99.9 ± 1.13	−0.1	3	100.6 ± 0.9	0.6	0.004	100.2 ± 0.17	0.2	5	98.8 ± 0.57	−1.2
	40	100.02 ± 0.93	0.02	5	99.6 ± 1.08	−0.4	0.007	100.06 ± 0.96	0.06	10	99.7 ± 0.86	−0.3
	55	99.16 ± 1.39	−0.84	8	99.2 ± 0.63	−0.8	0.010	101.4 ± 1.14	1.4	16	100.1 ± 0.74	0.1
ACR	6	99.6 ± 1.01	−0.4	3	100.4 ± 0.95	0.4	2	100.8 ± 1.6	0.8	3	98.24 ± 1.16	−1.76
	12	100.7 ± 1.42	0.7	6	100.3 ± 1.47	0.3	5	99.6 ± 1.05	−0.4	5	98.72 ± 0.66	−1.28
	20	99.7 ± 1.29	−0.3	10	100.2 ± 1.3	0.2	10	99.6 ± 1.06	−0.4	9	98.26 ± 0.39	−1.74

[a] Final concentration in μg/ml.
[b] Mean recovery ± S.D. for five determinations.
[c] Percentage relative error.

Table 3
Statistical comparison between the determination of CET, FEX, LOR and ACR by using the proposed and reference methods in authentic samples and pharmaceutical formulations

Preparation	BCP method, mean recovery ± S.D.	Eosin method, mean recovery ± S.D.	Fluorimetric method, mean recovery ± S.D.	CAA method, mean recovery ± S.D.	Reference method[a], mean recovery ± S.D.
(1) CET authentic sample	99.9 ± 1.3 $F = 3.4$ $t = 0.131$	99.9 ± 0.85 $F = 1.39$ $t = 0.12$	99.1 ± 1.7 $F = 5.57$ $t = 0.85$	100.26 ± 0.64 $F = 1.26$ $t = 1.06$	99.8 ± 0.7
Tomazine tablet, 10 mg per tablet	99.8 ± 1.28 $F = 2.41$ $t = 0.142$	100 ± 1.03 $F = 1.3$ $t = 0.32$	99.6 ± 1.5 $F = 2.7$ $t = 0.38$	101.3 ± 1.26 $F = 1.77$ $t = 2.03$	99.9 ± 0.9
(2) FEX authentic sample	100 ± 1.4 $F = 3.06$ $t = 0.27$	100.4 ± 1.5 $F = 3.5$ $t = 0.79$	99.0 ± 0.83 $F = 1.07$ $t = 0.39$	100 ± 0.85 $F = 1.12$ $t = 0.38$	99.8 ± 0.8
Telfast tablet, 120 or 180 mg per tablet	100.4 ± 1.4 $F = 3.06$ $t = 0.83$	100.4 ± 1.5 $F = 3.5$ $t = 1.87$	98.8 ± 0.73 $F = 1.07$ $t = 0.39$	100 ± 0.8 $F = 1.10$ $t = 0.38$	99.8 ± 0.8
(3) LOR authentic sample	99.8 ± 0.39 $F = 2.6$ $t = 0.89$	99.4 ± 1.4 $F = 4.7$ $t = 0.14$	99.9 ± 0.48 $F = 1.77$ $t = 1.11$	100 ± 1.06 $F = 2.74$ $t = 1.26$	99.5 ± 0.64
Claritine tablet, 10 mg per tablet	99.2 ± 0.82 $F = 1.07$ $t = 1.76$	100.3 ± 1.5 $F = 3.6$ $t = 0.26$	98.5 ± 1.18 $F = 2.17$ $t = 2.4$	98.9 ± 0.97 $F = 1.5$ $t = 2.1$	100.1 ± 0.79
Claritine syrup, 5 mg per 5 ml	98.3 ± 1.35 $F = 2.84$ $t = 1.7$	99.4 ± 1.19 $F = 2.21$ $t = 0.155$	98.38 ± 1.5 $F = 3.51$ $t = 1.47$	– – –	99.5 ± 0.8
(4) ACR authentic sample	99.8 ± 0.86 $F = 1.76$ $t = 0.41$	100.8 ± 0.84 $F = 1.67$ $t = 1.68$	100.4 ± 1.3 $F = 4.0$ $t = 0.61$	99.8 ± 1.18 $F = 3.29$ $t = 0.33$	100 ± 0.65
Semprex capsule, 8 mg per capsule	99.8 ± 1.2 $F = 2.9$ $t = 0.69$	99.2 ± 1.19 $F = 3.35$ $t = 1.65$	99.3 ± 1.39 $F = 4.570$ $t = 1.31$	99.8 ± 1.18 $F = 3.29$ $t = 0.66$	100.2 ± 0.65

Theoretical value $F = 6.39$ at the 95% confidence level. Theoretical value $t = 2.31$ at the 95% confidence level.
[a] A_{max} method.

References

[1] R.N. Brogden, D. Mc Tavish, Drugs 41 (1991) 927.
[2] J. Moncrieff, J. Chromatogr. Biomed. Appl. 121 (1992) 128–130.
[3] P. Van-Wensveen, J. Chem. Aust. 4 (2000) 17–19.
[4] M. Vogowska, M. Zajac, I. Muszalska, J. Chem. Anal. 45 (5) (2000) 681–688.
[5] J. Macek, P. Ptacek, J. Klima, J. Chromatogr. B: Biomed. 1–2 (1999) 231–235.
[6] E. Baltes, R. Coupez, L. Brouwers, J. Govert, J. Chromatogr. Biomed. Appl. 74 (1988) 149–155.
[7] R. Hsu, A.M. Au, J. Bull. Environ. Contam. Toxicol. 66 (2) (2001) 178–183.
[8] S.Y. Chang, F.R. Nelson, J.W.A. Findlay, L.C.E. Taylor, J. Chromatogr. Biomed. Appl. 89 (1989) 288–295.
[9] H.A. Squella, J.C. Sturm, M.A. Diaz, H. Pessoa, L. Numez Vergara, J. Talanta 43 (12) (1996) 2029–2035.
[10] M.M. Ghonam, M.M. Mabrouk, A.M. Hassanein, A. Tawfik, J. Pharm. Biomed. Anal. 25 (2001) 933–939.
[11] Z.H. Lin, J. Yaowu Fexi Zazhi 16 (1996) 53–56.
[12] A.F. ElWalily, M.A. Korany, A. ElGindy, M.F. Bedair, J. Pharm. Biomed. Anal. 17 (1998) 435–442.
[13] N. ElKousy, L.I. Bedawy, J. Pharm. Biomed. Anal. 20 (1999) 671–679.
[14] N.A. ElRagehy, A.M. Badawy, S.Z. Khateeb, J. Anal. Lett. 28 (1995) 2363–2378.
[15] K. Basavaih, Srilatha, M.J. Swamy, J. Talanta 50 (1999) 887–892.
[16] M.S. Prakash, M. Sundarapandian, S. Meena, M.S. Nagarajan, J. Indian Drugs 37 (2000) 211–212.

[17] H.H. Abdine, A.A. Gazy, S.M. Blaih, M.A. Korany, J. Talanta 43 (10) (1996) 1643–1648.
[18] H. Karam, N. El Kousy, M. Towakkol, J. Anal. Lett. 32 (1) (1999) 79–96.
[19] British Pharmacopoeia, HMSO, London, 1998, pp. 292–293.
[20] R.S. Bakry, O. Abdel Razak, A.F. ElWalily, S.F. Belal, J. Pharm. Biomed. Anal. 53 (1996) 325–329.
[21] N.A. El Ragehy, S.S. Abbas, S.Z. El Khateeb, J. Anal. Lett. 28 (10) (1995) 1799–1809.
[22] A.D. Thomas, Talanta 22 (1975) 865–869.
[23] A.D. Thomas, J. Pharm. Pharmacol. 28 (1976) 838–839.
[24] P. Job, Ann. Chim. 9 (1928) 113.