European Journal of Chemistry 3 (4) (2012) 421-425

European Journal of Chemistry

Journal homepage: www.eurjchem.com

Simultaneous determination of miconazole and hydrocortisone or mometasone using reversed phase liquid chromatography

Ramzia Ismail El-Bagary [a], Ehab Farouk Elkady [a],
Marwa Hosny Tammam [b], and Ayman Abo Elmaaty [b,*]

[a] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Cairo University, Cairo, 11562, Egypt*
[b] *National Organization For Drug Control and Research (NODCAR), Giza, 35521, Egypt*

**Corresponding author at: National Organization For Drug Control and Research (NODCAR), Giza, 35521, Egypt. Tel.: +2.0122.9257929; Fax: +2.02.24148452. E-mail address: dr_ayman3333@yahoo.com (A.A. Elmaaty).*

ARTICLE INFORMATION

Received: 24 July 2012
Received in revised form: 11 October 2012
Accepted: 12 October 2012
Online: 31 December 2012

KEYWORDS

Determination
Miconazole nitrate
Mometasone furoate
Hydrocortisone acetate
Pharmaceutical preparation
Reversed phase liquid chromatography

ABSTRACT

Two simple, rapid and precise reversed phase liquid chromatographic methods have been developed and validated for the simultaneous determination of miconazole nitrate in two binary mixtures, with hydrocortisone acetate (Mixture 1) and mometasone furoate (Mixture 2). For the two mixtures, chromatographic separation was carried out on a C18 column. For mixture 1, a mobile phase consisting of 2.22 mM sodium dihydrogen phosphate (Triethylamine 0.2%):acetonitrile (45:55, *v:v*) at a flow rate of 0.9 mL/min was used at ambient temperature. Quantitative determination of miconazole and hydrocortisone was achieved with UV detection at 215 and 245 nm, respectively. Linearity, accuracy and precision were found to be acceptable over the concentration range of 30-80 µg/mL for miconazole and 4-80 µg/mL for hydrocotisone. For mixture 2, a mobile phase consisting of acetonitrile:water (Triethylamine 0.2%) (70:30, *v:v*) at a flow rate of 0.9 mL/min was used at ambient temperature. Quantitative determinations of miconazole and mometasone were achieved with UV detection at 215 and 250 nm, respectively. Linearity, accuracy and precision were found to be acceptable over the concentration range of 10-200 µg/mL for miconazole and 2-60 µg/mL for mometasone. The optimized methods were proved to be specific, robust and accurate for the quality control of the cited drugs in pharmaceutical preparations.

1. Introduction

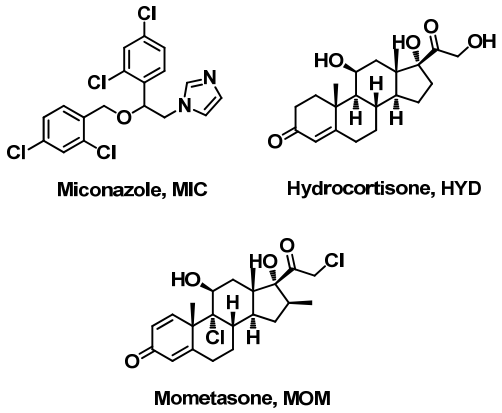
Miconazole; 1-[2,4-dichloro-β-(2,4-dichlorobenzyloxy) phenethyl]-imidazole (Scheme 1) is an antifungal agent with similar antimicrobial activity to ketoconazole [1]. It is used for treatment of superficial candidiasis, skin infections dermatophytosis and pityriasisversicolor [1].

Hydrocortisone; 11β,17α,21-trihydroxypregn-4-ene-3,20-dione (Scheme 1) is a principal glucocorticoid hormone [1,2]. It is produced by the adrenal cortex [2] and has been used clinically to treat skin problems such as rashes, eczema and others.

Mometasone; 9α,21-dichloro-11β,17-dihydroxy-16α-methylpregna-1,4-diene-3,20-dione (Scheme 1) is a corticosteroid used for its glucocorticoid activity [1].

This work is devoted for the simultaneous determination of miconazole in two binary mixtures which are available together in the form of creams.

Literature survey reveals that several analytical methods have been reported for the determination of miconazole nitrate (MIC) alone or in combinations including spectrophotometry [3-11], spectrofluorometry [4] and HPLC [12-30]. Besides, several analytical methods have been reported for the determination of hydrocortisone acetate (HYD) alone or in combination with other drugs including spectrophotometry [31-35], and HPLC [36-45]. MIC and HYD were simultaneously determined by TLC [46] and post column photochemical derivatization HPLC [47]. Also, several analytical methods have been reported for the determination of mometasone furoate (MOM) alone or in combination with other drugs by HPLC [48-52].



Scheme 1

Furthermore, literature survey reveals, MIC and HYD mixture was determined simultaneously by TLC and post column photochemical derivatization HPLC techniques [46,47] which is tedious and less applicable in quality control work. So, this work can develop quality control of miconazole and hydrocortisone in its semisolid dosage form by using simpler, available and less tedious methods. Thus, the aim of this work

ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online)
DOI:10.5155/eurjchem.3.4.421-425.667

was to develop simple and validated HPLC method applying C18 columns which are more commonly used. Besides, no previous method has been reported for the simultaneous determination of MIC and MOM. So such method was of interest using a mobile phase with simple composition (acetonitrile: water (Triethylamine 0.2%) (70:30, *v*:*v*)). So quality control of miconazole, hydrocortisone and mometasone was developed and sophisticated and that is one of the most important goals of my quality control work. New methods for simultaneous determination of two or more compounds in the same sample without previous chemical separation are always of interest. Due to wide application and use of RP-HPLC technique, the aim of this study was to develop and validate an alternative reversed-phase liquid chromatographic method for the determination of the binary mixture under investigation applying C18 columns.

2. Experimental

2.1. Instrumentation

A chromatographic system consisting of Agilent 1200 series (CA, USA); interface equipped with an Agilent quaternary pump G1311A, Agilent UV-visible detector G1314B, an Agilent manual injector G1328B equipped with (20 µL) injector loop, an Agilent degasser G1322A and thermo BDS hypersilC18 column (5 µm, 4.6 x 250 mm) was used. An Agilent syringe of LC 50 µL (CA, U.S.A.) and, ultrasonic processor; (Soniclean 120T, 220/240V, 50/60Hz, 60W, Thebarton SA, Australia) were also used.

2.2. Materials and reagents

The pharmaceutical grade MIC was supplied and certified by Camlin Fine Chemicals (Cairo, Egypt) to contain 99.70%. Pharmaceutical grade HYD was supplied and certified by EVA Pharma Company (Cairo, Egypt) with the purity of 99.85%. The pharmaceutical grade MOM with the purity of 99.80% and Mykotral H® Cream labeled to contain 20 mg MIC and 10 mg HYD for each 1 gram of cream were provided and manufactured by Sigma Pharmaceutical Industries Company (Cairo, Egypt). Elica M® Cream manufactured by Jamjom Pharmaceutical Industries Company was labeled to contain 20 mg of MIC and 1 mg of MOM for each 1gram of cream. Acetonitrile used was HPLC grade (Scharlau, Spain). Bi-distilled water was produced in-house (Aquatron Water Still, A4000D, UK). Membrane filters of 0.45 µm were purchased from Teknokroma (Barcelona, Spain). All other chemicals and reagents used were of analytical grade unless indicated otherwise.

2.3. Chromatographic conditions

Chromatographic separation was achieved on a thermo BDS hypercil C18 column (5 µm, 4.6 x 250 mm) applying an isocratic elution based on 2.22 mM sodium dihydrogen phosphate:acetonitrile (55:45, *v*:*v*) for mixture 1 and acetonitrile: water (70:30, *v*:*v*) for mixture 2 as mobile phases. Triethylamine (0.2%) was added to aqueous phase of the two mobile phases. The flow rate of mobile phase was set to 0.9 mL/min. The pH of the mobile phases was adjusted to 3.2 using *ortho*-phosphoric acid. The injection volume was 20 µL. Analyses were carried out at ambient temperature and detection was carried out at 215, 245 and 250 nm for MIC, HYD and MOM, respectively.

2.4. Standard solutions

Standard solutions of each of MIC, HYD and MOM were prepared by separately dissolving 20 mg of each drug in 100 mL mobile phase. Serial dilutions were prepared to get 1, 2, 4, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg/mL for MIC and 0.5, 1, 2, 4, 8, 10, 20, 40 and 60 µg/mL for HYD (Mixture 1) and getting 1, 2, 4, 8, 10, 40, 80, 120, 160 and 180 µg/mL for MIC and 1, 2, 4, 8, 10, 20, 30, 40 and 60 µg/mL for MOM (Mixture 2).

For mixture 1 further dilutions were made to obtain a concentration rang of 1-100 µg/mL for MIC and 0.5-60.0 µg/mL for HYD. For mixture 2 further dilutions were made to obtain a concentration rang of 1-180 µg/mL for MIC and 1-60 µg/mL for MOM.

2.5. Sample preparation

2.5.1. Mixture 1

One gram of Mykotral H® cream was accurately weighed and stirred in 80 mL of mobile phase with the aid of heat not exceeding 40 °C. The mixture was transferred quantitatively into a 100 mL volumetric flask and completed to volume with the mobile phase. The mixture was filtered to obtain a sample solution of concentration equivalent to 200 µg/mL for MIC and 100 µg/mL for HYD.

2.5.2. Mixture 2

One gram of Elica M ® cream was accurately weighed and stirred in 80 mL of mobile phase with the aid of heat not exceeding 40 °C. The mixture was transferred quantitatively into a 100 mL volumetric flask and completed to volume with the mobile phase. The mixture was filtered to obtain a sample solution of concentration equivalent to 200 µg/mL for MIC. The same procedure was repeated starting with 10 g of the cream to obtain 100 µg/mL for MOM.

2.6. Procedure

2.6.1. Linearity and repeatability

2.6.1.1. Mixture 1

Accurately measured aliquots of working standard solutions equivalent to 300-800 µg for MIC and 40-800 µg for HYD were transferred into two series of 10 mL volumetric flasks and completed to volume with mobile phase .The repeatability of the method was assessed by analyzing a laboratory prepared mixture containing 60 µg/mL for MIC and 30 µg/mL for HYD.

2.6.1.2. Mixture 2

Accurately measured aliquots of working standard solutions equivalent to 100-2000 µg/mL for MIC and 20-600 µg/mL for MOM were transferred into two series of 10 mL volumetric flasks and completed to volume the mobile phase. The repeatability of the method was assessed by analyzing a laboratory prepared mixture containing 120 µg/mL for MIC and 6 µg/mL for MOM (n = 6). The precision (R.S.D. %) for each compound was calculated.

A volume of 20 µL of each solution was injected in triplicates into the chromatograph under the specified chromatographic conditions described previously [53]. A calibration curve for each compound was obtained by plotting area under the peak (AUP) against concentration (C).

2.6.2. Assay of laboratory prepared mixtures, Mykotral H® and Elica M ® creams

2.6.2.1. Mixture 1

The procedure mentioned under Section 2.6.1 was repeated using laboratory prepared mixtures equivalent to 10-80 µg/mL MIC and 10-80 µg/mL HYD (Figure 1).

Table 1. Assay parameters and method validation obtained by applying HPLC method for the simultaneous determination of miconazole nitrate and hydrocortisone acetate or mometasonefuroate in mixtures *.

Item	Mixture 1		Mixture 2	
	MIC	HYD	MIC	MOM
Retention time, min	2.069	5.200	2.080	5.700
Wavelength of detection, nm	215	245	215	250
Range of linearity, µg/mL	30-80	4-80	10-200	2-60
Regression equation	y=16.4718x+48.212	y=51.1554x+6.599	y=17.1994x+29.186	y=63.5628x+12.888
Correlation coefficient (r^2)	0.9992	0.9995	0.9999	0.9995
LOD (µg/mL)	3.18	1.55	2.15	1.30
LOQ (µg/mL)	10.60	5.20	7.20	4.25
S_b	0.233	0.559	0.098	0.73
S_a	16.426	30.777	15.150	29.800
Confidence limit of the slope	16.4718±270.5	51.1554±1575.5	17.1994±260.0	63.5628±1894.0
Confidence limit of the intercept	48.2128±11.23	6.5996±3.69	29.1865±2.86	12.8888±9.40
Standard error of the estimation	9.74	37.44	16.70	34.60
Precision, Intra-day %R.S.D.	0.97	0.11	0.644	0.93
Precision, Inter-day %R.S.D.	0.67	0.73	0.76	0.35
Precision Drug in dosage form	100.1±1.49	100.214±1.01	100.12±1.30	100.13±1.46
Accuracy Drug in laboratory mixture	100.9±0.78	100.4±1.30	99.54±1.05	100.4±1.20
Accuracy Drug added	100.44±1.17	99.04±1.01	101.28±0.60	100.55±1.19

* a = slope, b = intercept, S_b = standard deviation of intercept, S_a = standard deviation of slope.

Table 2. Validation data for the proposed method.

Item	Mixture 1		Mixture 2	
	MIC	HYD	MIC	MOM
Number of theoretical plates	5443	12876	1728	2339
Resolution factor	-	19.53	-	11
Retention time	1.225	1.125	1.220	1.300
RSD% of 6 injections of Peak area (cm^2)	0.970	0.110	0.644	0.930
RSD% of 6 injections of Retention time (min)	0.086	0.040	0.660	0.370

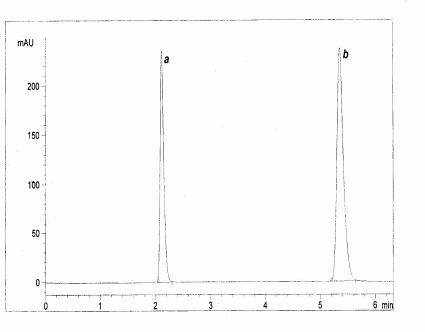


Figure 1. The chromatogram for lab prepared mixture of (**a**) (MIC) and (**b**) (HYD) (Mixture 1).

2.6.2.2. Mixture 2

The procedure mentioned under Section 2.6.1 was repeated using laboratory prepared mixtures equivalent to 20-190 µg/mL MIC and 5-60 µg/mL MOM (Figure 2).

For the determination of the examined drugs in Mykotral H® Cream and Elica M ® Cream, the sample solution prepared under Section 2.5 was diluted to prepare different solutions equivalent to 35-80 µg/mL MIC and 25-70 µg/mL HYD (Mixture 1) and 20-190 µg/mL MIC and 15-55 µg/mL MOM (Mixture 2) and injected in triplicate into the chromatograph.

3. Results and discussion

A literature survey reveals that MIC and HYD mixture was determined simultaneously by TLC and post column photochemical derivatization HPLC techniques which are tedious and less applicable in quality control work. Thus, the aim of this work was to develop simple and validated HPLC method applying C18 columns which are more commonly used.

A literature survey reveals no analytical work to separate MIC and MOM by HPLC techniques so, the aim of this work was to develop a new, simple, accurate and reproducible LC method for the simultaneous determination of MIC and MOM mixture in a semi-solid pharmaceutical formulation.

For both mixtures, statistical analysis of proposed and reference method reveals that no significant difference at probability of 0.05. So, ensuring accuracy and precision of proposed method (Table 1).

3.1. Method development

For the separation of the examined drugs, various reversed-phase C18 columns, isocratic mobile phase systems were attempted. The mobile phase composition and pH were studied and optimized.

3.1.1. Mixture 1

A satisfactory separation was obtained with a mobile phase composed of 2.22 mM sodium dihydrogen phosphate buffer, acetonitrile (45:55, *v*:*v*), adding triethylamine to the aqueous phase (0.2%). The mobile phase was adjusted to pH = 3.25 using *ortho*-phosphoric acid. At higher acetonitrile concentrations, separation was obtained but with excessive tailing for HYD peak. At lower acetonitrile concentrations, high retention times for peaks were obtained. At pH = 3.25, optimum resolution with reasonable retention times was observed (Table 2).

3.1.2. Mixture 2

A satisfactory separation was obtained with a mobile phase composed of acetonitrile and water (70:30, *v*:*v*) adding triethylmine to the aqueous phase (0.2%). The mobile phase was adjusted to pH = 3.2 using *ortho*-phosphoric acid. At higher acetonitrile concentrations, separation was obtained but with excessive tailing for MOM peak. At lower acetonitrile concentrations, MOM peaks are resoluted at higher retention time so much time is consumed. At pH = 3.2, optimum resolution with reasonable retention time was observed (Table 2).

Quantitative determination based on peak area was achieved with UV detection at 215, 245 and 250 nm for MIC, HYD and MOM respectively since such drugs give maximum peak absorbance at such wavelengths using UV scanning

spectrum. So, high sensitivity was obtained for the separated drugs.

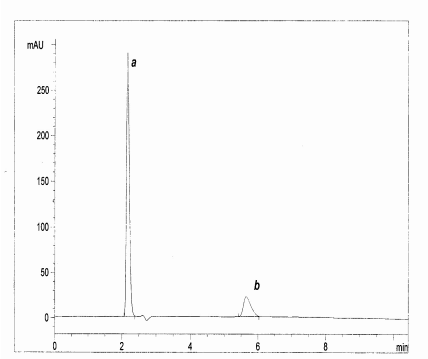


Figure 2. The chromatogram for lab prepared mixture of (**a**) (MIC) and (**b**) (MOM) (Mixture 2).

3.2. Validation of the method

3.2.1. Linearity

Linearity was studied for MIC, HYD and MOM. A linear relationship between area under the peak (AUP) and component concentration (C) was obtained. The regression equation for each drug was also computed (Table 1) in this study, six concentrations for each compound were used. The linearity of the calibration curves were validated by the high value of correlation coefficients (Table 1). The analytical data of the calibration curves including standard deviations for the slope and intercept (S_b and S_a) were summarized in Table 1.

3.2.2. Accuracy

Accuracy of the results was calculated by % recovery of laboratory prepared mixtures of 6 different concentrations of the MIC, HYD and MOM and also by standard addition technique for Mykotral H® and Elica M® cream. The results obtained including the mean of the recovery and standard deviations are displayed in (Table 1).

3.2.3. Precision

Precision was estimated by repeatability. The repeatability of the method was assessed by analyzing a mixture containing 60 and 30 µg/mL for MIC and HYD, respectively (n = 6) (Mixture 1), and analyzing a mixture containing 120 and 6 µg/mL for MIC and MOM (n = 6), respectively, (Mixture 2). The values of the repeatability (%R.S.D.) and inter-day and intra-day precision (using three different concentrations in triplicates for three days) for the three drugs in two methods are displayed in Table 1.

3.2.4. Specificity

Specificity is the ability of the analytical method to measure the analyte response in the presence of interferences. In the present work, the chromatograms of the samples were checked for the appearance of any extra peaks. No chromatographic interference from any of the excipients was found at the retention times of the examined drugs. In addition, the chromatogram of each drug in the sample solution is identical to the chromatogram received by the standard solution at the wavelengths applied. These results demonstrate that there was no interference from other materials in the pharmaceutical formulations and therefore confirm the specificity of the method.

3.2.5. Range

The calibration range was established through consideration of the practical range necessary, according to each compound concentration present in the pharmaceutical product, to give accurate, precise and linear results. The calibration range of the proposed HPLC method is given in Table 1.

3.2.6. Limits of detection and quantification

Limit of detection (LOD) representing the concentration of analyte at S/N ratio of 3 and limit of quantification (LOQ) at which S/N is 10 (Table 1). According to ICH recommendations [53], the approach based on the S.D. of the response and the slope were used for determining the detection and quantitative limits. The theoretical values were assessed practically and given in Table 1.

3.2.7. Robustness

Robustness is a measure of the method ability to remain unaffected by small variations in the method conditions and is an indication of the method reliability. Robustness was performed by deliberately changing the chromatographic conditions.

Variation of pH of the mobile phase by ±0.2 and its organic strength by ±2% did not have any significant effect on chromatograms.

The most important parameter to be studied was the resolution factor between the two peaks of MIC and HYD (Mixture 1) and MIC and MOM (Mixture 2). As can be seen in Tables 3-6, good values of the resolution factor were obtained for all these variations, indicating good robustness of the proposed LC method. The flow rate of the mobile phase was changed from 0.9 to 0.8 mL/min and 1.0 mL/min. The organic strength was varied by ±2% and pH was varied by ±0.2 units. The concentration of sodium dihydrogen phosphate buffer was varied by 2% (Mixture 1) (Table 6).

Table 3. The effect of flow rate of the mobile phase on resolution of peaks.

Item	Flow rate (mL/min)		
	0.8	0.9	1.0
Resolution factor MIC-HYD	20.54	19.52	19.19
Resolution factor MIC-MOM	11.2	11.24	11.16

Table 4. Influence of organic strength of the mobile phase on resolution of peaks.

Concentration vs. organic strength				
Mixture 1	Item, %	55	53	57
	Resolution factor MIC-HYD	19.61	19.87	18.42
Mixture 2	Item, %	68	70	72
	Resolution factor MIC-MOM	11.96	11.19	12.04

Table 5. Influence of pH of the mobile phase on resolution of peaks.

pH vs. resolution				
Mixture 1	Item, pH	3.15	3.25	3.35
	Resolution factor MIC-HYD	21.71	20.64	20.18
Mixture 2	Item, pH	3.10	3.20	3.30
	Resolution factor MIC-MOM	10.93	10.93	10.00

Table 6. Influence of concentration of sodium dihydrogen phosphate buffer on resolution of peaks.

Item (Mixture 1)	0.22 Mm	2.22 Mm	4.22 Mm
Resolution factor MIC-HYD	20.20	20.15	20.03

3.3. Statistical analysis of the results

A statistical analysis of the results was obtained by the proposed method. The reference methods were carried out by "SPSS statistical package version 11".

Table 7. Statistical analysis of the results obtained by the proposed LC method and the reference methods.

Statistical term	Mixture 1				Mixture 2			
	MIC		HYD		MIC		MOM	
	HPLC	Reference method **	HPLC	Reference method ***	HPLC	Reference method **	HPLC	Reference method ****
Mean	100.90	100.20	100.40	99.90	99.54	100.22	100.40	100.02
S.D.	0.780	0.509	1.300	0.645	1.052	0.510	1.200	0.542
R.S.D.	0.770	0.508	1.300	0.646	1.056	0.508	1.200	0.542
N	6	6	6	6	6	6	6	6
Variance	0.610	0.259	1.690	0.416	1.100	0.260	1.440	0.293
t-value	1.84 (2.228) *		0.85(2.228) *		1.45 (2.228) *		0.72(2.228) *	
F-value	2.35(5.05) *		4.06(5.05) *		4.26(5.05) *		4.91(5.05) *	

* Figures in parentheses are the corresponding values for theoretical t- and F-values at p = 0.05.
** Reference method for MI using HPLC method according to U.S.P34 [54].
*** Reference method for HY using UV spectrophotometry method according to B.P. 2011 [55].
**** Reference method for MOM using HPLC method according to U.S.P 34 [54].

The significant difference between groups was tested by T-test and F-test at p = 0.05 as shown in Table 7. The test ascertained that there was no significant difference among the methods.

4. Conclusion

The proposed HPLC method provides simple, accurate and reproducible quantitative analysis for the simultaneous determination of MIC and HYD or MOM in creams. This method was validated as per ICH guidelines [53]. The proposed method is suitable for the quality control determination of the cited drugs in ordinary laboratories

References

[1]. Reynolds, J. E. F. The extra pharmacopeia, 37th edition, Volume A, 2011.
[2]. O'Neil, M. J. The Merck Index 14th edition, An Encyclopedia of Chemicals, Drugs and Biologicals, 2006.
[3]. Erk, N.; Altun, M. L. *J. Pharm. Biomed. Anal.* **2001**, *25(1)*, 115-122.
[4]. Khashaba, P. Y.; El-Shabouri, S. R.; Emara, K. M.; Mohamed, A. M. *J. Pharm. Biomed. Anal.* **2000**, *22(2)*, 363-376.
[5]. Goger, N. G.; Gokcen, L. *Anal. Lett.* **1999**, *32(13)*, 2595-2602.
[6]. Wrobel, K.; De-la-Garza-Rodriguez, I. M.; Lopez-de-Alba, P. L.; Lopez-Martinez, L. *J. Pharm. Biomed. Anal.* **1999**, *20(1-2)*, 99-105.
[7]. El-Shabouri, S. R.; Emara, K. M.; Khashaba, P. Y.; Mohamed, A. M. *Anal. Lett.* **1998**, *31(8)*, 1367-1385.
[8]. Erk, N. *STP. Pharma. Sci.* **1996**, *6(37)*, 312-315.
[9]. Chen, B. *Zhongguo Yiyao Gongye Zazhi* **1993**, *24(7)*, 318-319.
[10]. Cavrini, V.; Di-Pietra, A. M.; Gatti, R. *J. Pharm. Biomed. Anal.* **1989**, *7(12)*, 1535-1543.
[11]. Cavrini, V.; Di-Pietra, A. M.; Raggi, M. A. *Pharm. Acta. Helv.* **1981**, *56(6)*, 163-165.
[12]. Huang, Q. X.; Yu, Y. Y.; Tang, C. M.; Peng, X. Z. *J. Chromatogr. A.* **2010**, *1217(21)*, 3481-3488.
[13]. Moradi, M.; Yamini, Y.; Vatanara, A.; Saleh, A.; Hojati, M.; Seidi, S. *Anal. Methods* **2010**, *2(4)*, 387-392.
[14]. DeZan, M. M.; Camara, M. S.; Robles, J. C.; Kergaravat, S. V.; Goicoechea, H. C. *Talanta* **2009**, *79(3)*, 762-767.
[15]. Ali, I.; AboulEnein, H. Y.; Gaitonde, V. D.; Singh, P.; Rawat, M. S. M.; Sharma, B. *Chromatographia* **2009**, *70(1/2)*, 223-227.
[16]. Zhong, W.; Tang, X.; Wu, Q. J. *Yaowu Fenxi Zazhi* **2006**, *26(9)*, 1305-1307.
[17]. Cakar, M.; Popovic, G.; Agbaba, D. *J. Aoac. Int.* **2005**, *88(5)*, 1544-1548.
[18]. Cirilli, R.; Ferretti, R.; Gallinella, B.; La-Torre, F.; La-Regina, G.; Silvestri, R. *J. Sep. Sci.* **2005**, *28(7)*, 627-634.
[19]. Aboul-Enein, H. Y.; Ali, I. *J. Pharm. Biomed. Anal.* **2002**, *27(3-4)*, 441-446.
[20]. Aboul-Enein, H. Y.; Ali, I. *Chromatographia* **2001**, *54(3-4)*, 200-202.
[21]. Aboul-Enein, H. Y.; Ali, I. *FreseniusJ. Anal. Chem.* **2001**, *370(7)*, 951-955
[22]. Indrayanto, G.; Widjaja, S.; Sutiono, S. *J. Liq. Chromatogr. Relat. Technol.* **1999**, *22(1)*, 143-152.
[23]. Morin, N.; Guillaume, Y. C.; Rouland, J. C. *Chromatographia* **1998**, *48(5-6)*, 388-394.
[24]. Han, J.; Zeng, H. J.; Tang, H. F. *Yaowu Fenxi Zazhi* **1997**, *17(1)*, 9-11.
[25]. Kobylinska, M.; Kobylinska, K.; Sobik, B. *J. Chromatogr. B: Biomed. Appl.* **1996**, *685(1)*, 191-195.
[26]. Roychowdhury, U.; Das, S. K. *J. Aoac. Int.* **1996**, *79(3)*, 656-658.
[27]. Zhang, S.; Luo, S.; Zhang, F.; Cai, H.; Yin, W. *Yaowu Fenxi Zazhi* **1993**, *13(3)*, 198-199.
[28]. Tyler, T. A.; Genzale, J. A. *J. Assoc. Off. Ana. Chem.* **1989**, *72(3)*, 442-444.
[29]. Selinger, K.; Matheou, D.; Hill, H. M. *J. Chromatogr.* **1988**. *434,* 259-264.
[30]. Hosotsubo, H. *Chromatographia* **1988**, *25(8)*, 717-720
[31]. Blanco, M.; Coello, J.; Iturriaga, H.; Maspoch, S.; Villegas, N. *Analyst* **1999**, *124(6)*, 911-915.
[32]. Bonazzi, D.; Andrisano, V.; Gatti, R.; Cavrini, V. *J. Pharm. Biomed. Anal.* **1995**, *13(11)*, 1321-1329.
[33]. Nikolic, K.; Medenica, M.; Bogavac, M.; Arsenijevic, L. *Farmaco* **1991**, *46(4)*, 623-626.
[34]. Walash, M. I.; Zakhari, N. A.; Rizk, M.; Toubar, S. *Farmaco* **1987**, *42(3)*, 81-90.
[35]. Rizk, M.; Zakhari, N.; Toubar, S.; Walash, M. I. *Acta Pharm. Fenn.* **1984**, *93(3)*, 129-134.
[36]. Zuo, Z. H.; Tang, S. F. *Yaowu Fenxi Zazhi* **2010**, *30(8)*, 1516-1519.
[37]. Chauhan, V.; Conway, B. *Chromatographia* **2005**, *61(11-12)*, 555-559.
[38]. Zuo, Z. H.; Zhou, J. M.; Zuo, W. U. *Yaowu Fenxi Zazhi* **2004**, *24(4)*, 420-421.
[39]. Hajkova, R.; Solich, P.; Dvorak, J.; Sicha, J. *J. Pharm. Biomed. Anal.* **2003**, *32(4-5)*, 921-927.
[40]. Lemus-Gallego, J. M.; Perez-Arroyo, J. *Anal. Bioanal. Chem.* **2002**, *374(2)*, 282-288.
[41]. Ku, Y. R.; Wen, K. C.; Ho, L. K.; Chang, Y. S. *Yaowu Shipin Fenxi* **1999**, *7(2)*, 123-130.
[42]. Reubsaet, J. L. E.; Vieskar, R. *J. Chromatogr. A.* **1999**, *841(2)*, 147-154.
[43]. Valenta, C.; Janout, H. *J. Liq. Chromatogr.* **1994**, *17(5)*, 1141-1146.
[44]. Bhounsule, G. J.; Gorule, V. S.; Patil, G. V. *Indian Drugs* **1992**, *29(13)*, 594-597.
[45]. Hailey, D. M.; Lea, A. R. *J. Assoc. Off. Ana. Chem.* **1981**, *64(4)*, 870-874.
[46]. Mousa, B. A.; El-Kousy, N. M.; El-Bagary, R. I.; Mohamed, N. G. *Chem. Pharm. Bull.* **2008**, *56(2)*, 143-149.
[47]. Di-Pietra, A. M.; Andrisano, V.; Gotti, R.; Cavrini, V. *J. Pharm. Biomed. Anal.* **1996**, *14(8-10)*, 1191-1199.
[48]. Shaikh, S.; Muneera, M. S.; Thusleem, O. A.; Tahir, M.; Kondaguli, A. V. *J. Chromatogr. Sci.* **2009**, *47(2)*, 178-183.
[49]. Sahasranaman, S.; Tang, Y. F.; Biniasz, D.; Hochhaus, G. *J. Chromatogr. B.* **2005**, *819(1)*, 175-179.
[50]. Wulandari, L.; Sia, T. K.; Indrayanto, G. *Liq. Chromatogr. Relat. Technol.* **2003**, *26(1)*, 109-117.
[51]. Teng, X. W.; Foe, K.; Brown, K. F.; Cutler, D. J.; Davies, N. M. *J. Pharm. Biomed. Anal.* **2001**, *26(2)*, 313-319.
[52]. Spangler, M. *Supelco. Rep.* **1994**, *13(2)*, 12-13.
[53]. The European Agency for the Evaluation of Medical Products, ICH Topic Q2B Note for Guidance on Validation of Analytical Procedures, Methodology GPMP/ICH/281/95, 1996.
[54]. The United States Pharmacopeial Convention, Thirty-Fourth Revision, NF29, 2011.
[55]. British pharmacopeia. The stationary office, medical and pharmaceutical substances, London, 2011.