

**HPLC, densitometric and spectrophotometric  
methods for the simultaneous determination  
of colchicine and probenecid in their binary  
mixture**

---

**ABSTRACT**

**Aim:** To develop methods with complete validation according to ICH guidelines and to be applied for the determination of both drugs in laboratory prepared mixtures and in pharmaceutical formulations

**Study design:** High performance liquid chromatography (HPLC), densitometric and different spectrophotometric methods (zero order, derivative ratio, ratio difference and mean centering) are developed for simultaneous determination of colchicine and probenecid in their combined pharmaceutical formulation.

**Methodology:** High performance liquid chromatography separation is developed using C18 column and methanol: ammonia (100: 1.5 v/v) as a mobile phase. The densitometric method based on the separation of both drugs using chloroform: methanol: ethyl acetate: water: ammonia (7: 5:2.5:0.5:0.5 by volume) as mobile phase and scanning  $\lambda$  at 254 nm. Zero order determination is based on measurement of colchicine absorbance at 349 nm. The first derivative ratio of peak amplitudes at 367 nm & at 290.4 nm and the ratio difference with the amplitude difference between (385 nm and 362.4 nm) and (270 nm and 255 nm) for colchicine and probenecid, respectively are developed for determination of both drugs. Mean centering determination of probenecid is developed by measurement at 279 nm using 3.6  $\mu\text{g/mL}$  of colchicine as a divisor.

**Results:** HPLC method was applied over the concentration ranges of 1.0-45.0  $\mu\text{g/mL}$  & 0.5-30.0, while densitometric method was linear over the concentration 0.15-0.6 & 0.15-0.45  $\mu\text{g / band}$  and

spectrophotometric methods were linear over the concentration ranges 10.00-55.0 & 3.6-20.0 µg/mL for colchicines and probenecid, respectively.

**Conclusion:** Novel, simple and accurate method for the determination of colchicine and probenecid simultaneously in their binary mixture.

*Keywords: colchicine, probenecid, HPLC, densitometry, spectrophotometry.*

## 1. INTRODUCTION

Colchicine; (S)-N-(5,6,7,9-tetrahydro-1,2,3,10-tetramethoxy-9oxobenzol[a]heptalen-7-yl) acetamide) is an alkaloid contained in various species of colchicum and in other genera [1]. It is used in the relief of acute gout probably by reducing the inflammatory reaction to urate crystals [2].

Probenecid; (4-(Dipropylsulfamoyl) benzoic acid) [1] is a uricosuric agent used for the treatment of hyperuricemia associated with chronic gout, hyperuricemia caused by diuretic therapy and as adjunct to some antibacterial to reduce their renal tubular excretion [2]. It is used in combination with colchicine to treat chronic gouty arthritis when complicated by frequent recurrent acute attacks of gout. It inhibits the absorption of urate in the proximal convoluted tubule, thus increasing the urinary excretion of uric acid and decreasing serum urate levels [3].

The literature review revealed that numerous techniques have been applied for the analysis of probenecid in a single dosage form such as HPLC [4-7], TLC [8-9], spectrophotometric [10-13], capillary electrophoresis [14-15] and spectrofluorimetry [16]. Also, various techniques were reported for the single determination of colchicine as HPLC [17-20], TLC [21-23], spectrophotometry [24-25] and electrochemistry [26-28]. Only two chromatographic methods [29, 30] have been reported for the determination of both drugs in binary mixtures. Notably, the only reported HPLC method [29] needs tedious sophisticated instrumentation and no published spectrophotometric method was developed for the determination of both drugs simultaneously until now. Therefore, it was valuable to develop simple and fast procedures which can be applied in quality control laboratories for the determination of both drugs simultaneously. In this work, spectrophotometric methods based on first derivative ratio, ratio difference and mean centring was first applied for determination of both drug in binary mixture. Also two chromatographic methods, reversed-phase HPLC and densitometric methods are reported for the quantification of both drugs. These methods are applied to determine both drugs in commercial pharmaceutical formulations and in laboratory prepared mixtures.

## 2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

### 2.1. INSTRUMENTATION

The chromatographic HPLC (Agilent 1200 series, Germany) apparatus consists of an Agilent pump, equipped with a variable wavelength detector. The separation was performed using kromasil C18 column (250 mm × 4 mm) and the mobile phase "methanol: ammonia (100:1.5 v/v)" was pumped at a flow rate 1 mL/min after filtration and sonication. The detection wave length was 246 nm.

Sample for densitometric method was applied by an automatic sample applicator provided with 100 µL syringe to TLC plates precoated with Silica Gel60F254, 10x20 cm (Merck, Germany) and scanning by COMAG TLC scanner combined with WINCATS software (CAMAG, Switzerland) with scanning speed of 20 mm/ s.

A dual-beam UV-visible spectrophotometer [Shimadzu, Japan] model UV-1601 PC. Shimadzu UV- PROB version 2.32 and MATLAB®, version 7.0.124704 were used to process the absorbance, the derivative spectra and mean centring. The sample solution were recorded in 1 cm quartz cells against solvent blank over the range 200–400 nm.

### 2.2. MATERIALS AND REAGENTS

Colchicine and probenecid were kindly supplied by Pharaonia Pharmaceutical Co. and October Pharm Co. Cairo, Egypt, respectively. Their purities were found to be 99.7% and 99.5% for colchicine and probenecid, respectively referred to the reported methods [7, 19]. Goutyless® tablet labelled to contain 0.5 mg colchicine and 500 mg probenecid and was purchased from October Pharma, Cairo, Egypt. Ethanol and methanol, chloroform and ethyl acetate were of chromatographic grade (Fisher scientific, USA). Water was doubly distilled.

## 2.3. STANDARD SOLUTIONS

### 2.3.1. Stock standard solutions

Stock standard solutions of colchicine and probenecid (1 mg/mL) were prepared in methanol for (HPLC and TLC methods) and in ethanol for spectrophotometric method.

### 2.3.2. Working standard solutions

For HPLC. Working standard solutions (0.1 mg/ml) were prepared in methanol and standard solutions of colchicine and probenecid containing concentration ranges of 5.00- 300.00 and 10.00 – 450.00 µg/ mL were prepared in methanol, respectively.

For densitometry. Working standard solutions (0.1 mg/ml) were prepared in methanol. Standard solutions equivalent to (75.0-225.0 µg/ mL) and (75.0-300.0 µg / mL) for colchicine and probancid were prepared in methanol.

For spectrophotometry. Working standard solutions (0.1 mg/ml) were prepared in ethanol. Standard solutions containing concentration range of (36.0-200.0 µg/ mL) and (100.0-550.0 µg/ mL) for colchicine and probenecid, respectively were prepared in ethanol.

### 2.3.3. Laboratory prepared mixtures

Different aliquots within calibration ranges from working colchicines solution in methanol (0.1 mg/ mL) were mixed with aliquots within calibration ranges of working probenecid solution (0.1 mg/ mL) and volumes were completed with suitable solvents for each method.

## 2.4. SAMPLE SOLUTION

**Colchicine** - 5 Goutyless® tablets were weighed and crushed to a fine powder. An amount of powder equivalent to 1 mg of colchicine and 1000 mg of probenecid was dissolved in 30 ml of water for HPLC and TLC and ethanol for spectrophotometric method. After sonication for 15 min the volume was then made up to the mark in a 50 ml volumetric flask with the same solvent. Filtration was carried out using syringe filter to labelled concentration of 20 µg / mL colchicine. Further dilution was done with methanol for HPLC and TLC or ethanol for spectrophotometric method.

**Probenecid** - An amount of fine powder equivalent to 0.5 mg of colchicine and 500mg of probenecid was dissolved in 70 ml of methanol for HPLC &TLC or ethanol for spectrophotometric method. The solution was sonicated for 15 min, made up to the mark in a 100- ml volumetric flask with the same solvent and filtered through filter paper to reach a labelled concentration of 5 mg/ mL probenecid . Further probenecid dilution was carried out with the corresponding solvent to obtain a solution a labelled to contain 100 µg / mL probenecid .

## 2.5. PROCEDURES

**HPLC method**, 100 µL injections from each solution were chromatographed as under conditions described previously" 2.1". The calibration curve was constructed by plotting the peak area against the corresponding drug concentration and the regression equation was evaluated.

**Densitometric method**, 20 µL of each solution was applied to a TLC plate (20 × 10 cm) and spotted as bands of 6 mm width, 5 mm interval and 2 cm from the bottom. The plate was developed for distance of 9 cm in chromatography tank presaturated with the mobile phase of chloroform: methanol: ethyl acetate: water: ammonia (7: 5: 2.5: 0.5: 0.5 by volume) for 30 min, then it was scanned at 254nm. The calibration curve representing the recorded area under the peak against drug concentration in µg /spot was plotted and the regression equation was evaluated.

**Spectrophotometric method**, The spectra of the prepared standard solutions were scanned from 200 - 400 nm and stored in the computer. For zero order method : The absorbance of colchicine at 349 nm was plotted against the corresponding drug concentration and the regression equation was evaluated. For first derivative ratio (1DR): The stored spectra of colchicine were divided by the spectrum of (10 µg/mL) of probenecid and the first derivative of the ratio spectrum (1DR) was recorded using  $\Delta\lambda = 8$  and scaling factor 1. Spectra of probenecid were divided by the spectrum of (3.6 µg/ml) of colchicine and the first derivative of the ratio spectrum (1DR) was obtained using  $\Delta\lambda = 4$  and scaling factor= 1. The peak amplitude at 367.0 nm for colchicine and at 290.4 nm for probenecid were plotted against drug concentration for derivative ratio method (1DR). Ratio difference (RD) was obtained by measuring the amplitude difference between (385nm and 362.4 nm) for colchicine and between (270.0nm and 255.0 nm) for probenecid and the

108 difference was plotted against their corresponding drug concentration. For mean centering (MCR): The obtained spectra  
 109 of probenecid were mean cantered at 279.0 nm using (3.6 µg/mL) colchicine as divisor. Value obtained were plotted  
 110 against probenecid concentration.

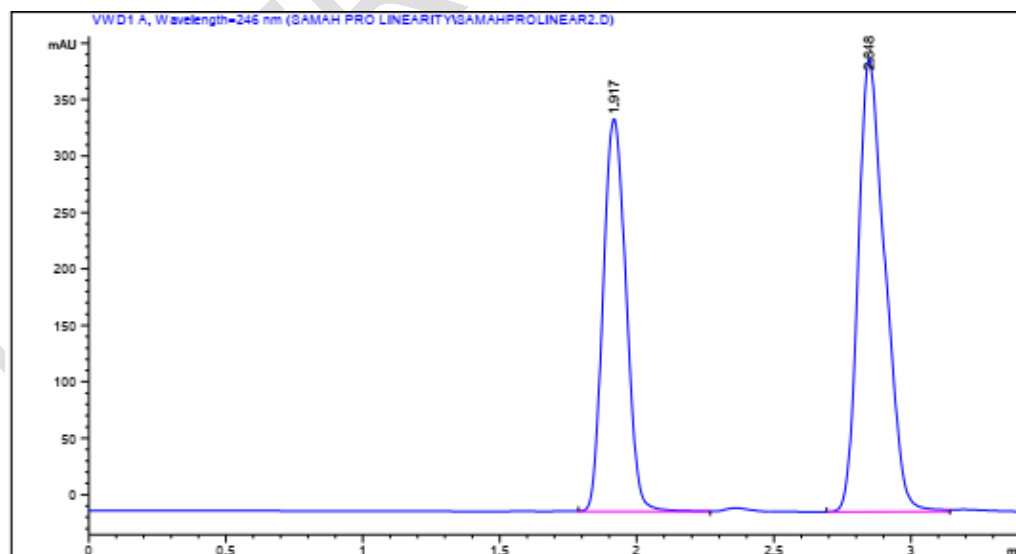
### 111 3. RESULTS AND DISCUSSION

#### 112 HPLC method

113 As there was only one very tedious HPLC method was reported [29] for the determination of colchicine and  
 114 probenecid in mixture so this reversed phase HPLC method was developed to provide simple and fast procedure for the  
 115 analysis of the mixture in quality control laboratories. Different mobile phase systems composed of variable solvents with  
 116 different ratios were tested and the best resolution was achieved by using methanol: ammonia (100:1.5 v/v) as a mobile  
 117 phase which was pumped with flow rate 1ml/min. The best separation with the good tailing factor of the peaks and highest  
 118 no of theoretical plates was achieved by using kromasil C18 column (250 x 4 mm) and detection wavelength at 246 nm.  
 119 By using the selected chromatographic conditions, retention times were found to be 1.917 and 2.848 for: probenecid and  
 120 colchicine, respectively, (Figure 2) and the results of system suitability is shown at Table 1. These retention times are  
 121 shorter than retention times for the reported one " 2.4 nm and 4.3 nm for colchicine and probenecid, respectively [29].  
 122 Table (1): System suitability data for HPLC for determination of probenecid and colchicine.

Parameters	Obtained value		
	probenecid	Colchicine	Reference value
Retention time	Rt=1.917	Rt=2.848	
capacity factor (K')	0.92	1.86	0.5-10 is acceptable
Selectivity factor ( $\alpha$ )	2.01		> 1
Resolution factor(R)	5.53		R >2
tailing factor (T)	1.130	1.282	Not more than 2
symmetry	0.84	0.64	(0.5-1)
Number of plates	2446	3931	The higher the more efficient the column

124

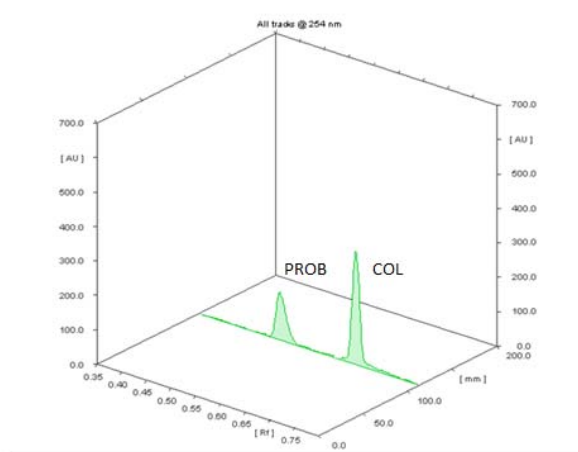


125

**Figure 2: HPLC chromatogram of laboratory mixture of probenecid  $R_t = 1.917$  and Colchicine  $R_t = 2.818$  at 246 nm.**

### Densitometric method

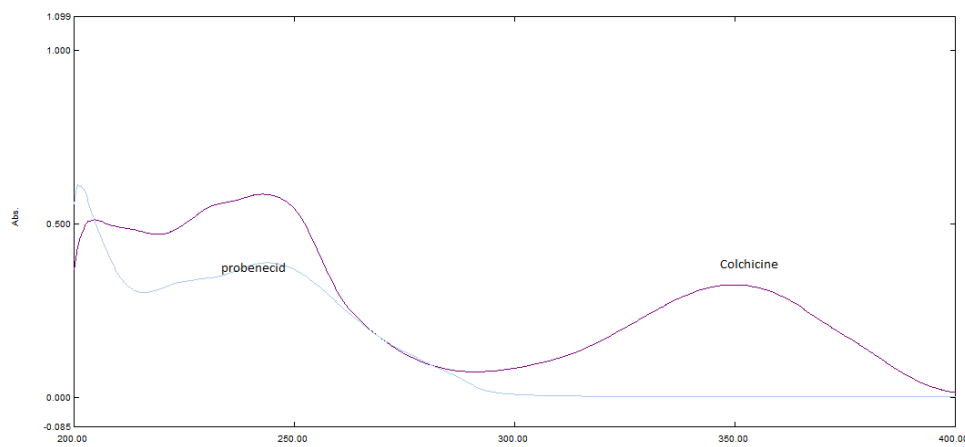
The TLC-Densitometric technique was successfully applied for simultaneous determination of colchicine and probenecid mixture. Developing systems of different composition and ratios were tested; as chloroform: acetone, ethylacetate: methanol: ammonia, chloroform: methanol and chloroform : methanol: ethyl acetate. Upon using mobile phase composition of (chloroform: methanol: ethyl acetate: water 7:5:2.5:0.5 by volume), the valuable separation was recorded but with slight closed  $R_f$ . Addition of ammonia to the mobile phase (chloroform: methanol: ethyl acetate: water: ammonia 7:5:2.5:0.5:0.5 by volume) gave well separated symmetry bands at  $R_f$  0.53 and 0.69 for colchicin and probenecid, respectively, (Figure 3). Different scanning wavelengths were tested (246 nm, 254nm and 348nm) and 254 nm was found to be the most suitable wave length for the detection of both drugs rather than the reported method [30], which required two wavelengths to be measured.



**Figure 3: Densitometric chromatogram of mixture of colchicine and probenecid**

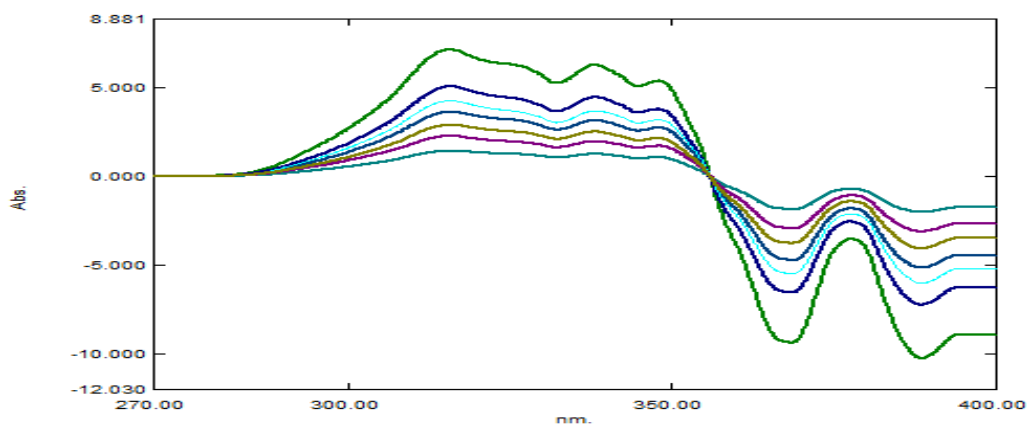
**Spectrophotometric methods** As no spectrophotometric method was reported for the determination of two drugs simultaneously up till now, development of several spectrophotometric methods is significant for fast and easy determination of mixture in quality laboratories.

**-Zero order-The** zero-order absorbance spectra of colchicine and probenecid showed obvious overlapping but the extended part in colchicine spectra allowed its determination at 349 nm in presence of probenecid, Figure (4).

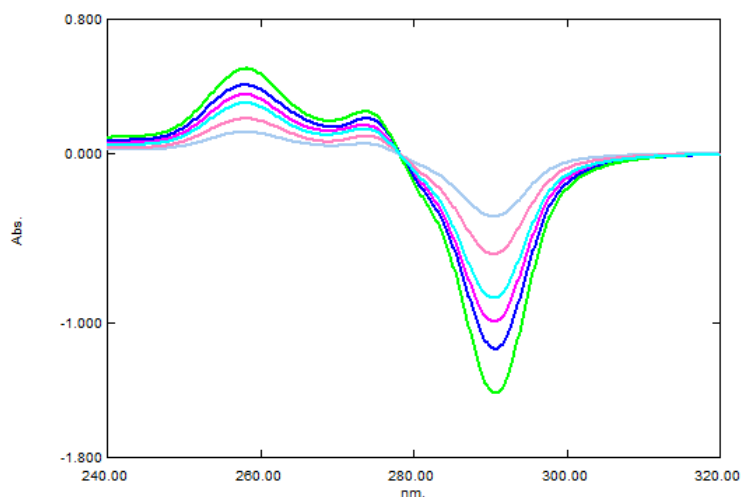


**Figure 4: Absorption spectra of probenecid and colchicine in ethanol**

**-First derivative ratio (1DR)** -This method depends on division of the mixture's spectrum by the spectrum of one of the two component. Then the derivative ratio spectrum of that mixture will be independent on that divisor and the other component can be determined with no interference [31]. Different parameters were studied such as concentration of divisor, wavelength and the wavelength increment over which the derivative of the ratio spectra derivative is obtained ( $\Delta\lambda$ ). The sharpest and best peak amplitude were achieved using  $\Delta\lambda=8$  for colchicine and  $\Delta\lambda=4$  for probenecid. Different concentrations of colchicine (3.6, 10 and 20  $\mu\text{g/mL}$ ) and of probenecid (10, 30 and 55 $\mu\text{g/mL}$ ) were tested as a divisor, the minimum noises in ratio spectra and the best recoveries were shown at the concentrations 3.6  $\mu\text{g/mL}$  of colchicine and 10  $\mu\text{g/mL}$  of probenecid. There was a reasonable linearity at wavelengths 315, 338, 367, 377, 388 nm for colchicine and 258, 270, 275, 290.4 nm for probenecid but the best recoveries were at 367.2 nm and 290.4nm for colchicine and probenecid, respectively, figure (5a,5b).

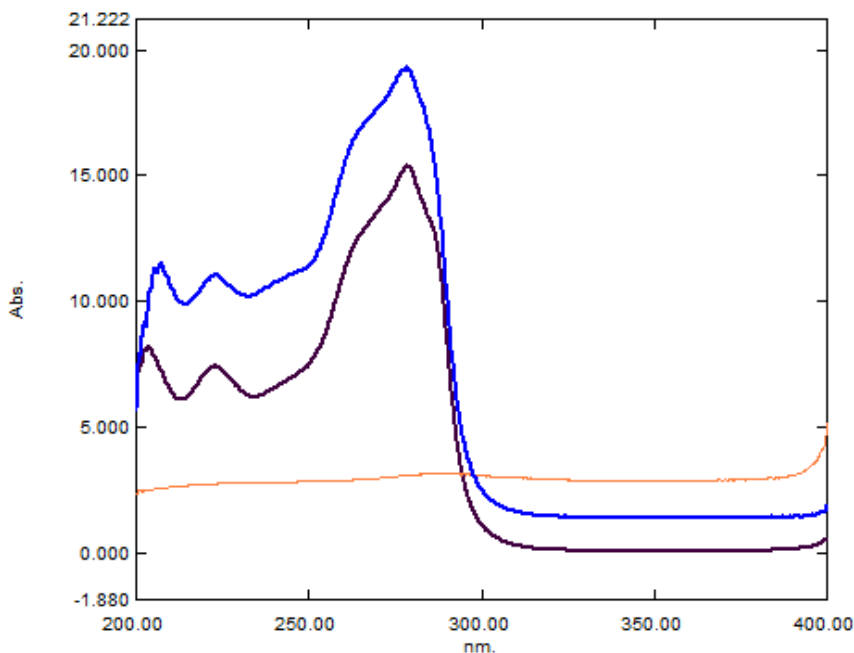


**Figure (5a): First derivative of ratio spectra of colchicine (3.60-20.00  $\mu\text{g/ml}$ ) using 10.00  $\mu\text{g /ml}$  probenecid as divisor and ethanol as blank.**



**Figure (5b): First derivative of ratio spectra of probenecid (10.00-55.00 µg/mL) using 3.60 µg /ml colchicine as divisor and ethanol as blank.**

**-Ratio difference (RD):** It has the ability of solving severely overlapped spectra without prior separation with high degree of simplicity, accuracy and reproducibility [31]. It can be carried out at any two wavelengths throughout the whole ratio spectrum, where no contribution of the overlapped component in the amplitude difference at any wavelength couples [32]. As shown in Figure (6a, 6b), (385 nm-352.4 nm) and (270 nm -255nm) were the chosen as amplitude differences for colchicine and probenecid, respectively where linear correlations against the corresponding concentrations of both drugs were obtained.



**Figure (6a): Ratio spectra of probenecid (---) (55.00 µg/ml), colchicine (----)(10.00 µg/ml), their mixture using 3.6 µg/ml colchicine as divisor and ethanol as blank.**

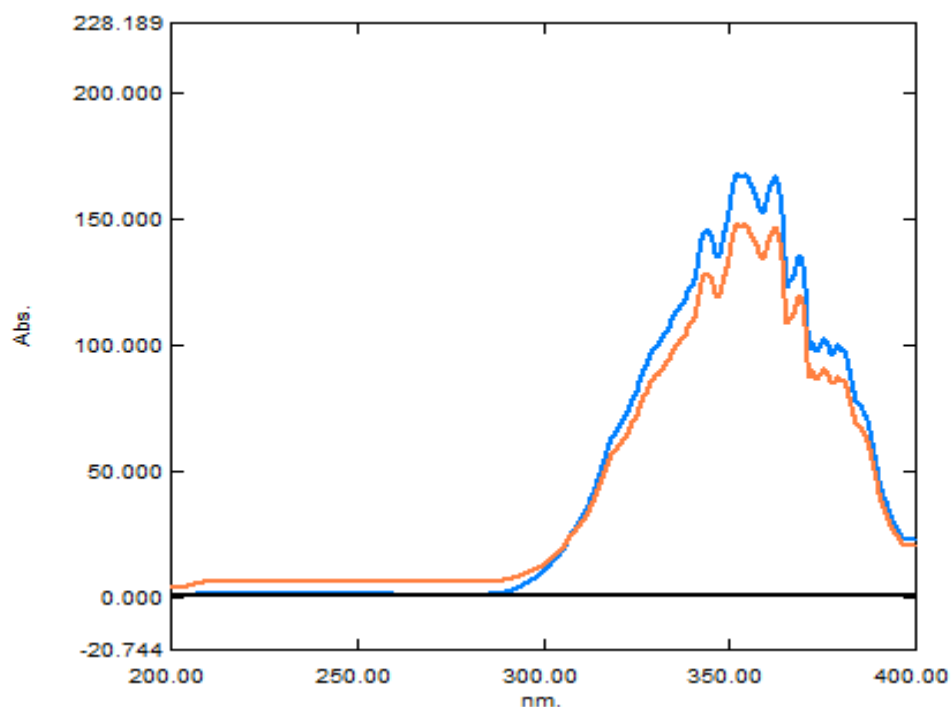


Figure (6b): Ratio spectra of probenecid (55.00 µg/ml), colchicine (10.00 µg/ml), their mixture of the same concentration using 10 µg/ml of probenecid as divisor and ethanol as blank.

**-Mean centering:** The ratio spectra are obtained, after which the constant is removed by mean centering of the ratio spectra [33]. Probenecid concentration is determined by measuring the amplitude of mean centered peak at 279 nm, figure (7).

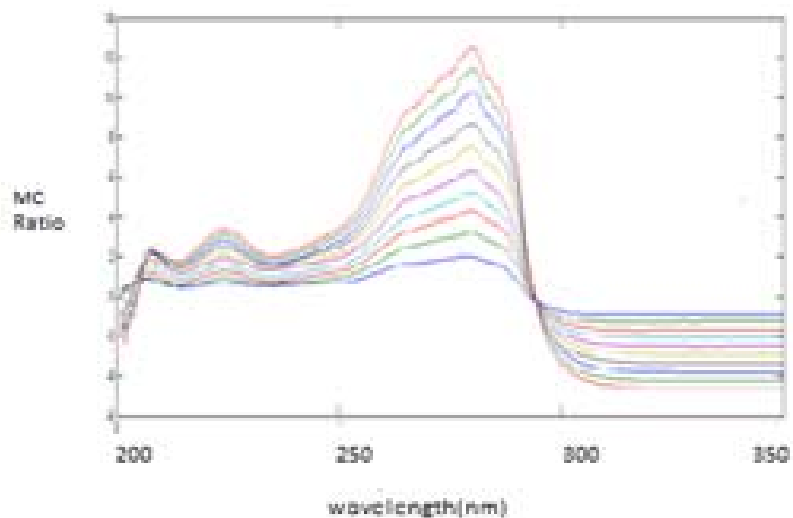


Figure (7): Mean centered ratio spectra of probenecid (10.00-55.00 µg/ml) using 3.6.0 µg/ml of colchicine as a divisor and ethanol as blank.

## Method validation

Validation of the methods was carried out according to the ICH recommendation [34]

### Linearity



Good linearity was obtained over the concentration ranges of 1.0-45.0 µg/mL & 0.5-30.0 " HPLC method", 0.15. 0-0.6 & 0.15-0.45 µg / band "densitometric method" and 10.00-55.0 & 3.6-20.0 µg/mL "spectrophotometric methods" for colchicines and probenecid, respectively. Regression parameters were summarized in Table (2).

**Table (2): Regression and assay validation parameters by the proposed methods.**

	Probenecid					Colchicine				
	HPLC	DR1	RD	MC	TLC	HPLC	DR	RD	ZO	TLC
<b>λmax (nm)</b>	244	290.4	270-255	279	254	246	367	385-352.4	349	254
<b>Linearity range (µg/ml)</b>	1-45	10-55	10-55	10-55	0.15-0.60*	0.5-30	3.6-20	3.6-20	3.6-20	0.15-0.45*
<b>Slope</b>	264.52	0.0294	0.1031	0.2337	5725.1	413.64	0.5468	9.7642	0.0322	8056.7
<b>Intercept</b>	77.409	-0.0337	-0.191	-0.326	495.07	52.266	0.0178	1.7992	0.0014	985.98
<b>Correlation coefficient (r<sup>2</sup>)</b>	0.9999	0.9995	0.9998	0.9998	0.9994	0.9998	1.0	0.9999	0.9999	0.9992
<b>Accuracy (mean±SD)</b>	99.12±2.74	99.26±0.95	98.34±0.29	99.50±1.65	100.11±2.37	100.16±0.99	99.89±0.62	99.44±0.49	100.36±0.83	98.19±0.15
<b>Precision (RSD%)</b>	0.97	0.91	1.37	0.78	0.72	0.29	0.09	0.47	0.19	1.05
<b>Interday</b>	0.95	1.21	1.40	1.86	1.43	0.45	0.93	1.49	0.72	1.72
<b>Intraday</b>										

\*is µg/band

#### **Accuracy**

The accuracy of the proposed methods was studied by analysis of three different concentrations of each pure sample drug within the linearity ranges and the concentrations were calculated from the corresponding regression equations. Further assessment of accuracy is done by application of standard addition technique. It expressed as mean R% and RSD%, Tables (2) showed acceptable results for accuracy.

#### **Precision**

Repeatability and intermediate precision were determined by analyzing three different concentrations of probenecid and colchicine three times on a single day and on three consecutive days, by the proposed methods. Intraday RSD% was ranged between 0.72, 1.37 and 0.09, 1.05 while inter day RSD% range was 1.21, 1.86 and 0.45, 1.72 for colchicines and probenecid, Table (2).

#### **Selectivity**

Selectivity of proposed methods was evaluated by the determination of different synthetic laboratory prepared mixtures containing different ratios of probenecid and colchicine within the linearity range. Satisfactory recoveries ranged between 98.68±1.96 and 100.96±1.24 for colchicine and 99.05±0.82, 100.41±2.16 for probenecid were obtained, Table (3).

**Table 3. Determination of probenecid and colchicine in their mixtures using the proposed methods**

		Probenecid (A)					Colchicine (B)				
(A)	(B)	HPLC	DR1	RD	MC	TLC	HPLC	DR	RD	ZO	TLC
15.3	1		102.5	97.22	98.39	-		102.98	101.27	98.43	-
5.5	1		98.18	102.08	99.72	-		99.03	98.94	100.12	-
2.75	1		101.25	98.32	99.42			99.61	98.94	99.90	-
1	1	100.85	-	-	-	100.35	98.36	100.26	100.60	101.68	100.45
1	2	100.39	-	-	-	99.2	99.83	102.01	99.41	100.83	101.13
2	1	101.63	-	-	-	104.78	97.83	100.73	99.83	101.98	96.31
3.67	1		97.30	101.82	97.75			102.08	97.92	101.77	-
4	1		98.78	102.43	99.20	99.49		100.97	97.85	100.71	97.02
11.1 1	1		99.11	100.58	99.84	-		100.93	101.28	100.55	-
4	3		-	-	-	101.35		-	-		99.12
1	3		-	-	-	96.02		-	-		98.35
90	1	98.99					102.86				
3	2	97.58					99.53				
<b>Mean <math>\pm</math>SD</b>		99.89 $\pm$ 1.61	99.52 $\pm$ 1 .96	100.41 $\pm$ 2.16	99.05 $\pm$ 0 .82	100.2 $\pm$ 2 .87	99.68 $\pm$ 1.96	100.96 $\pm$ 1.24	99.56 $\pm$ 1.30	100.66 $\pm$ 1.11	98.73 $\pm$ 1 .89

**Analysis of pharmaceutical formulation**

The proposed methods were applied for the determination of the cited drugs in their combined dosage form to study the interference effect of the added excipients. As the ratio of probenecid and colchicine in Goutyless® tablet is 1000:1 thus, the determination is carried out by preparing two separate dilutions for each drug. Although separate formulation dilutions were prepared but the very high probenecid concentration still making a problem on TLC plate and HPLC column. This was overcome by changing the solvent used in the first dilution. Water was used as solvent where probenecid was insoluble and colchicine was very soluble. Table (4) showed recoveries percent results, the represented data support good recoveries of two standards from mixture of tablets diluted in 2 solvents. These data prove simultaneous determination of two drugs in mixture and valuable application of standard addition technique. Statistical comparison of the results obtained by the proposed methods and a reported one [29] showed that both calculated t and F ratio were less than the theoretical ones indicating that there was no significant difference between two methods, Table (4).

**Table 4: Determination of probenecid and colchicine in pharmaceutical formulation by the proposed methods and comparison with the manufacture method (43)**

Probenecid	HPLC	TLC	DR	RD	MC	Reported(43)
Mean $\pm$ SD	98.18 $\pm$ 2.00	100.32 $\pm$ 1.5	99.37 $\pm$ 2.04	99.09 $\pm$ 1.94	100.71 $\pm$ 1.28	100.28 $\pm$ 2.25
variance	4.01	2.25	4.17	3.78	1.65	5.07
Test number	5	5	5	5	5	5
t-test	1.56	0.04	0.67	0.89	0.37	-
F-ratio	0.79	0.44	0.82	0.74	0.32	-
Standard addition	100.72 $\pm$ 1.92	103.05 $\pm$ 0.57	98.79 $\pm$ 1.73	100.32 $\pm$ 1.93	98.97 $\pm$ 1.27	-

Colchicine	HPLC	TLC	DR	RD	ZO	Reported(43)
Mean $\pm$ SD	99.29 $\pm$ 1.58	98.02 $\pm$ 1.76	100.28 $\pm$ 1.86	99.19 $\pm$ 1.51	102.14 $\pm$ 0.73	99.61 $\pm$ 2.48
Variance	2.51	3.11	3.46	2.26	0.53	6.15
Test number	5	5	5	5	5	5
t-test	0.24	1.17	0.48	0.33	2.19	-
F-ratio	0.41	0.51	0.56	0.37	0.09	-
Standard addition	99.80 $\pm$ 1.41	101.18 $\pm$ 0.53	100.79 $\pm$ 0.65	100.43 $\pm$ 1.47	101.06 $\pm$ 1.75	

The theoretical t- and F- values at  $P=0.05$  were 2.31 and 6.39; respectively.

#### 4. CONCLUSION

Novel, simple and accurate spectrophotometric methods for the determination of colchicine and probenecid simultaneously in their binary mixture was developed. Moreover an alternative RP- HPLC and densitometric methods were developed to overcome the disadvantages of the reported HPLC method which required pre-extraction. The proposed methods are simple, accurate, precise, specific, and low cost; Hence, they can be used for routine analysis.

#### REFERENCES

- 1- Parkway T , Rockville MD. The United States Pharmacopeial Convention, USP. 39 th. Ed, USA, 2016.
- 2- Sweetman,SC. The Complete Drug Reference,39th Ed., Pharmaceutical Press,London,UK, 2017.

- 3- <https://pubchem.ncbi.nlm.nih.gov/compound/Probenecid#section=Top> .
- 4- Patel SC, Chauhan PP, Patel NM, Shah Sardar SK. Novel isocratic RP-HPLC for simultaneous multicomponent analysis of amoxicillin and Probenecid in pharmaceutical formulation. *IJIPLS*.2014;4(5): 28-36.
- 5- Nilam P, Paranjape DP. RP-HPLC method development and validation for simultaneous estimation of cefadroxil and Probenecid in synthetic mixture. *IJUPBS*.2014;3(3):560-571.
- 6- Harle RK, Cowen T. Determination of Probenecid in serum by high-performance liquid chromatography. *Analyst*.1978;103:492-496.
- 7- Jin-fang S, Yan-ling S, Xin Y. HPLC determination of ampicillin and Probenecid in their compound capsules. *J. of Pharma. Anal.*2004;3:411- 413.
- 8- Dhaneshwar SR, Kadam SS, Sirisha V; Development and validation of a HPTLC method for simultaneous estimation of cefuroxime axetil and Probenecid. *J Pharm Sci*.2004;66:278 - 282.
- 9- Al-Badr AA, El-Obeid HA. Probenecid, in *Analytical Profiles of Drug Substances*. Academic Press. 1981: 639.
- 10- Jain D, Jain DK, Trivedi P. Simultaneous Spectrophotometric Determination of Amoxycillin And Probenecid In Tablet Dosage Form. *Indian J. Pharm. Sci.*1998;60 (5):318 -320.
- 11- Kurian T, Kurien J. Simultaneous multicomponent spectrophotometric analysis of ampicillin and Probenecid in pharmaceutical formulation by derivative spectroscopy. *H.J.D.Med*.2011;3(2): 57 - 61.
- 12- Maheta PS, Patel PR, Parmar RR, Modasiya MMK, Dushyant A. Development and validation of derivative spectroscopic method for simultaneous estimation of cefadroxil and Probenecid. *IJPSN*.2014;7(1): 2350 - 2355.
- 13- Kumar R, Nain P, kaur J. Development and validation of uv-visible spectroscopic method for estimation of Probenecid in tablet dosage form. *IJAR*.2016; 4(5):212 - 219.
- 14- Sun H, Wu Y. Effective separation and simultaneous determination of cefamandole and Probenecid in body fluids by capillary zone electrophoresis with salicylic acid as an internal standard. *Anal. Methods*.2013; 5:6017 - 6022.
- 15- Sirén H, Shimmo R, Sipola P, Abenet S, Riekkola ML. Capillary electrophoresis of diuretics and Probenecid in methanol. *J Chromatogr A*.2008;(11): 1198 - 1199.
- 16- Cunningham RF, Israil ZH, Dayton PG; New spectrophotofluorometric assay for Probenecid. *J Pharm Sci*.1978; 67(3): 434 - 436.
- 17- Nirmala K, Raju RR. A novel method development for validation and detection of colchicine drug by RP-HPLC. *RJC*;5(1): 106 - 111.
- 18- Joshi SA, Jalalpure SS, Kempwade AA, Peram MR. Development and Validation of HPLC Method to Determine Colchicine in Pharmaceutical Formulations and its Application for Analysis of Solid Lipid Nanoparticles. *CPA*.2018;14(1): 76 - 83.
- 19- Samanidou VF, Sarantis GA, Papadoyannis IN. Development and validation of a rapid HPLC method for the direct determination of colchicine in pharmaceuticals and biological fluids. *J Liq Chromatogr R T*.2006;29(1/4): 1- 13.
- 20- Abdulbaqi IM, Darwis Y, Khan N, Loh GOK, A simple (HPLC–UV) method for the quantification of colchicine in bulk and ethosomal gel nano-formulation and its validation. *Int J Pharm Pharm Sci*.2017; 9(7):72 - 78.
- 21- Sarg, TM, El-Domiaty MM, Bishr MM. Thin-layer chromatographic scanner, spectrophotometric and high-performance liquid chromatographic methods for the determination of colchicine. *ANALYST*.1989;114(5):575 - 578.
- 22- Hadad GM, Badr JM, El-Nahriry K, Hassanean HA. Validated HPLC and HPTLC Methods for Simultaneous Determination of Colchicine and Khellin in Pharmaceutical Formulations. *J Chromatogr Sci*.2013;51(3): 258-265.
- 23- Bodoki E, Oprean R, Vlase L, Tamas M, Sandulescu R. Fast determination of colchicine by TLC-densitometry from pharmaceuticals and vegetal extracts. *J Pharm Biomed Anal*.2005;37:971 - 977.
- 24- Ajage RK, Kasture VS. Validated UV spectroscopic method for the estimation of three marker compounds in marketed polyherbal ayurvedic formulation. *Der Pharmacia Lettre*.2014;6(3):160 - 166.
- 25- Verma P, Patial A. Development of UV spectrophotometric method for estimation of colchicine in phosphate buffer saline pH 6.4. *IRJP*.2012;3(2):87 - 89.
- 26- Zhang H. Electrochemistry and voltammetric determination of colchicine using an acetylene black-dihexadecyl hydrogen phosphate composite film modified glassy carbon electrode. *j.bioelechem*.2006;68(2):197 - 201.
- 27- Wang F, Zhou J, Liu, Y, Wu S, Song G, Baoxian Y. Electrochemical oxidation behavior of colchicine on a graphene oxide-Nafion composite film modified glassy carbon electrode. *RSC*.2011;136: 3943 - 3949.
- 28- Stanković DM, Švorc L, Mariano JF, Ortner A, Kalcher K; Electrochemical Determination of Natural Drug Colchicine in Pharmaceuticals and Human Serum Sample and its Interaction with DNA. *Electroanalysis*.2017;29:2276 - 2281.
- 29- Lo W, G. M. Krause; Simultaneous Determination of Probenecid and Colchicine in Solid Dosage Form by Reversed Phase High Performance Liquid Chromatography; *Drug Dev. Ind. Pharm*.1987; 13(1): 57 - 66.
- 30- Mohamed AI, Omar MA, Hammad M, Mohamed AA. Validated thin-layer chromatographic method for alternative and simultaneous determination of two anti-gout agents in their fixed dose combinations. *Open Chemistry*.2018; 16: 496–510.

- 318 31- Lotfy M, Saleh SS, Hassan NY, Elgizawy SM. A Comparative Study of the Novel Ratio Difference Method versus  
319 Conventional Spectrophotometric Techniques for the Analysis of Binary Mixture with Overlapped Spectra.  
320 AJAC.2012; 3:761 - 769.
- 321 32- Elzanfaly ES, Saad AS , Abd-Elaleem AB. Simultaneous determination of retinoic acid and hydroquinone in skin  
322 ointment using spectrophotometric technique (ratio difference method). SPJ.2012;20(3) : 249 - 253.
- 323 33- Lotfy HM , Saleh SS. Recent development in ultraviolet spectrophotometry through the last decade. Int J Pharm  
324 Sci.2016;8(10):40 - 56.
- 325 34- International Conference on Harmonization (ICH). ICH Harmonized Tripartite Guideline. Topic Q2 (R1). Validation  
326 of Analytical Procedures: Text and Methodology; Geneva, Switzerland; 2005
- 327