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Development and Validation of Different Chromatographic Methods for Determination of Two Hypouricemic Drugs in their Combined Dosage Form

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Abstract: *Two sensitive, selective and precise chromatographic methods have been developed, optimized and validated for Allopurinol (ALP) and Benzbromarone (BENZ) determination in their pure form, laboratory prepared mixtures and pharmaceutical dosage form. The first developed method was based on thin layer chromatographic (TLC) combined with densitometric determination of the separated spots. The separation was achieved using silica gel 60F_{254} TLC plates. The mobile phase used was chloroform: methanol (9.2:0.8, v/v) and UV detection at 240nm.Good correlations were obtained between the integrated peak area of the studied drugs and their corresponding concentrations in different ranges. The second developed method was based on the high-performance liquid chromatography with ultraviolet detection, by which the proposed components were separated using Zorbax C_{18} column using a mobile phase consisting of sodium acetate buffer (pH=4.5, adjusted with acetic acid): acetonitrile: triethylamine (50:50:0.5, by volume) maintaining the mobile phase flow rate at 1mLmin^{-1}with UV detection at 260nm. Different parameters affecting the suggested methods were optimized for maximum separation of the cited components. System suitability parameters of the two developed methods were also tested. Validation of the methods has been carried out according to USP requirements and ICH guidelines, accuracy, precision and repeatability were found to be within the acceptable limits. The results obtained by TLC-Densitometric and RP-HPLC methods were statistically compared with those obtained by the reported RP-HPLC method and no significant difference was found regarding both accuracy and precision.*

Keywords: *Allopurinol, Benzbromarone, TLC-Densitometric, RP-HPLC.*

1. INTRODUCTION

Allopurinol(ALP), is (1,5Dihydro-4H-Pyrazolo[3,4-d]pyrimidin-4-one) [1],Fig.1, It is an official drug in British (BP) and United States (USP) Pharmacopoeias[1,2] which is used for treatment of gout and hyperuricaemia [3]. It is a xanthine oxidase inhibitor [4-7], which prevents the oxidation of hypoxanthine to xanthine and xanthine to uric acid [8].Thus results in the reduction of urate and uric acid concentrations in plasma and urine.

Benzbromarone (BENZ), is (3,5-dibromo-4-hydroxyphenyl)-(2-ethyl-3- benzofuranyl) methanone [1], Fig.2, It is an official drug in British(BP) Pharmacopoeia[1] which is used as a hypouricaemic drug. It increases the excretion of uric acid by blocking renal tubular reabsorption and thus reduces plasma concentrations and increases the excretion of uric acid [9, 10].

Combination of ALP and BENZ has the advantages of greater therapeutic effect than with either drug alone [11].This combination causes manifold reduction in uric acid concentrations in plasma and urine as compared to double dose of the individual drug when used alone[12]. Also, this combination helps to decrease the dose of each active ingredient, and as a result, decreases side effects of each component if given separately in high doses [13].

Reviewing the literature in hand, only one report has been published for determination of the studied mixture which depended on measuring BENZ using zero order spectra at its λ_{max}= 356 while ALP was determined by using (^{2}D) amplitudes at 281.4nm or by measuring the amplitudes

of the second derivative of the ratio spectra curves (2DD) at 282.4nm after using a standard spectrum of 8 μgmL^{-1}BENZ as a divisor [11].Also, the studied drugs have been analyses by TLC-Densitometric method using acetone: chloroform: NH_3 (5:4:0.01, by volume) as a developing system and by RP-HPLC method using phosphate buffer pH=4.0-acetonitrile-methanol (50:30:20, by volume) as a mobile phase [11].

Due to the pharmaceutical importance of this combination and from the previous literature review, it is important to develop simple, sensitive, time saving and cost effective methods for simultaneous analysis of the studied drugs which can be used for their quality control analysis.

The developed work aimed to develop and validate two chromatographic methods, TLC-Densitometric method and RP-HPLC method, for simultaneous determination of both ALP and BENZ. The developed TLC-Densitometric method has the advantages over the published one on using one wavelength for detection of the two studied components while the published one depends on using two wavelengths. Also, it is pH independent while the published method depends on using ammonia by 0.01 in the developing system. Therefore, the developed TLC-Densitometric method is highly sensitive and selective using one developing system and one wavelength for determination of ALP and BENZ together. On the other hand, the developed RP-HPLC has many advantages over the published one[11] on using sodium acetate buffer which gives the optimum conditions for the best separation of the peaks of the two studied components together with sharp peaks appears after acceptable retention time with high resolution and hence signal to noise ratio is enhanced.

Therefore, the developed RP-HPLC method has the advantage of being more selective and sensitive than the published one [11]. The proposed methods have been optimized and validated as per the International Conference on Harmonization(ICH) guidelines ICH, and were found to comply with the acceptance criteria [14].

2. EXPERIMENTAL

2.1. Instruments

2.1.1. TLC- Densitometric Method

1. CAMAG TLC scanner 3 S/N 130319 withwin CATS software.

The following requirements are taken into consideration:

-Source of radiation: deuterium lamp.

-Scan mode: absorbance mode.

-Slit dimension: 3mm*0.45 mm.

-Scanning speed: 20 mms^{-1}.

-Output: chromatogram and integrated peak area.

2. Linomat 5 autosampler (Switzerland).
3. CAMAG microsyringe (100µL).
4. Precoated silica gel aluminum plates 60 F254, ALLUGRAM® SIL G/UV 254 (Macherey.Nagel, Germany) 20×20 cm with 0.2mm thickness.
5. Sonix TV ss-series ultrasonicator (USA).

2.1.2. RP-HPLC Method

The HPLC system (Agilent Chem. Station HPLC B.04.03) consisted of a quaternary system with automatic injection facility, loop capacity 20µL, UV-visible detector and LC solution version 1.25 software. The column used was Zorbax C_{18} (250×4.6mm). The detector was adjusted at 245nm.

2.2. Materials

2.2.1. Pure standards

Standard ALP and BENZ were kindly supplied by GLOBAL NAPI PHARMACEUTICALS, 2nd Industrial Zone, 6th of October city. Egypt. With claimed purity of 98.36% and 98.43% according to a reported HPLC method.

2.2.2. Pharmaceutical dosage form

Alloben® tablets (100/20) (B.N.100251) labeled to contain 100mg Allopurinol+ 25mg Benzbromarone and were manufactured by GLOBAL NAPI PHARMACEUTICALS, 2nd Industrial Zone, 6th of October city. Egypt.

2.2.3. Chemicals and solvents

All chemicals and solvents used throughout this work were of analytical grade and were used without purification.

-Chloroform and methanol (El-Nasr Pharmaceutical Chemicals Co.Abu-Zabaal, Cairo, Egypt).

-Deionized water purchased from (SEDICO Pharmaceuticals Co., Cairo, Egypt).

-Acetonitrile, methanol, sod acetate buffer, acetic acid and triethylamine were of HPLC grade (Sigma-Aldrich® Chemie GmbH, Germany).

2.2.3. Standard Stock Solutions

Standard stock solutions of both ALP and BENZ of 1 $mgmL^{-1}$ concentration were prepared in either methanol (for TLC method) or in acetonitrile (for HPLC method).

2.2.4. Standard Working Solutions

Working standard solutions were made of ALP and BENZ (0.1 mgmL-1).Appropriate dilutions were made from the stock standard solutions of both Alp and Benz to prepare their corresponding working standard solutions.

2.3. Procedure

2.3.1. Chromatographic Conditions

2.3.1.1. Tlc-Densitometric method

Chromatographic separation was performed using precoated silica gel 60 F_{254} TLC aluminium plates (20×10cm).The plates were pre-washed with methanol and activated at 100ºC for 15 minutes prior to samples application. Samples were applied in the form of bands (4 mm length, 8.9 mm spacing and 15 mm from the bottom edge of the plate).Linear ascending development was performed in a chromatographic tank previously saturated with chloroform: methanol (9.2: 0.8, v/v) for half an hour at room temperature to a distance of about 80mm.The developed plates were air dried and then scanned at 240nm.

2.3.1.2. RP-HPLC Method

Chromatographic analysis was performed in isocratic mode with sodium acetate buffer: acetonitrile: triethylamine (50:50:0.5, by volume pH=4.5 with acetic acid) as a mobile phase delivered at 1 mLmin-1, injection volume 20µL and scanning at 240nm at room temperature. The run time was 10 min and the total peak area was used to quantify each of the studied drugs.

2.3.2. Construction of the calibration curves

2.3.2.1. Tlc-Densitometric method

Accurate volumes equivalent to (0.3-3 µgml-1) for both ALP and BENZ were separately transferred from their respective standard working solutions (0.1mgmL-1) applied in triplicates on the prewashed TLC plates in the form of bands and the procedure under chromatographic conditions was followed. The area under peak was then recorded and calibration curve for each

ALP and BENZ was constructed by plotting the mean integrated peak area ×10-4 versus the corresponding concentration.

2.3.2.2. RP-HPLC method

Working standard solutions (0.1mgmL-1) of ALP and BENZ were further diluted with acetonitrile to obtain dilutions in the range of (1-50µgmL-1) of each of ALP and BENZ, respectively. Triplicate 20µL injections were made for each prepared solution and chromatographed. The peak areas ×10-6 were plotted against the corresponding concentrations to obtain the calibration graph for each component.

2.3.3. Application to pharmaceutical dosage form

The content of ten tablets each of Alloben® (100/20) tablets were separately weighed and then finely powdered. Accurate amount of each powdered tablets equivalent to 1mg of ALP and BENZ were separately weighed, dissolved in 75mL methanol and sonicated for about 15 minutes. The prepared solutions were then filtered, transferred quantitatively to four separate 100-mL volumetric flasks and the volume was then completed to the mark with methanol. Appropriate dilutions of the prepared solutions were made to prepare their working solutions (0.1mgmL-1) and the developed method was then followed.

3. DISCUSSION

3.1. Tlc-Densitometric Method

The main task of this work is to establish a sensitive, selective and accurate Tlc-Densitometric method for determination of ALP and BENZ in bulk powder and pharmaceutical formulations using the developing system and detection at single wavelength with satisfactory precision for good analytical practice [15].

3.2. Method Development and Optimization

Different experimental conditions, such as developing system composition, band width and scanning wavelength, were optimized to provide accurate, precise and reproducible chromatographic separation. Different developing systems of different compositions were tried in order to obtain optimum separation. Satisfactory separation was achieved upon using chloroform: methanol (9.2: 0.8, v/v). This system was found to give compact sharp symmetrical spots for the two components with suitable R_f values at 240nm.**Fig**.

Different band widths were tested in order to obtain sharp and symmetrical separated peaks. The optimum band width chosen was 4mm and inter-space between bands was 8.9mm.Moreover, Different scanning wavelengths were tried such as 220, 254 and 240nm. At 240nm maximum sensitivity, sharp, symmetrical peaks with minimum noise were obtained and good sensitivity for both ALP and BENZ with a single plate scan.

3.3. RP-HPLC Method

A simple, accurate and selective RP-HPLC has been investigated and validated for quantitative analysis of ALP and BENZ. The LC procedure was optimized with a view to develop a quantitative method in a convenient time analysis with high quality separation of the two proposed components [16]. The chromatographic operational conditions were selected by considering the peak resolution and retention times of the first and the last eluted components.

3.4. Method Development and Optimization

For successful method validation, preliminary tests were performed with the objective to select adequate and optimum condition [17].

Parameters affecting the efficiency of the chromatographic separation has been tested and optimized in a trial to obtain the maximum separation of the cited components as follows:

3.5.1. The Stationary Phase

The stationary phase has a very important role that leads to the best separation [18]. Different stationary phase were tried (C_{18}, C_8 and CN columns) but Zorbax C_{18} gave the most acceptable peak shape for the studied drugs.

3.5.2. The mobile phase

The mobile phase composition and proportion, pH of the buffer and its type and the effect of triethylamine were also illustrated. Different mobile phases have been tested to achieve the best chromatographic separation among the studied drugs such as methanol: water (70: 30, v/v), methanol: phosphate buffer (75: 25 and 25:75, v/v), acetonitrile: water (60: 40 and 40: 60, v/v), acetonitrile: methanol (50:50, v/v), acetonitrile: phosphate buffer (50: 50, v/v). Using the last system; resulted in symmetrical peak for BENZ, but unfortunately broad peak with tail for ALP. Effect of triethanolamine (TEA) was also tested in different concentrations beginning with 0.1, 0.2, 0.3, 0.4 and 0.5 mL. Using the last concentration gave the most acceptable peaks of both ALP and BENZ. Effect of pH on the separation and peak symmetry was also tested ranging from (pH = 3 – 6.5), at pH = 3, ALP eluted as a peak with shoulder while BENZ retained on column, While at pH=6.5, ALP eluted as a tailed peak and hence bad resolution, and BENZ eluted in a peak with a shoulder. And lately at pH=4.5, ALP appeared as some wise good peak but still with a small tail, while BENZ appeared in a good symmetrical peak.

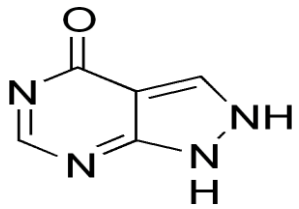
Studying the effect of different types of buffers plays an important role in achieving the best chromatographic conditions for the best separation of the two cited components. By trying sodium phosphate buffer we noticed that ALP eluted as a tailed peak, and BENZ appeared as a good symmetrical peak but after 10min as a retention time. While using sodium borate buffer, ALP eluted as a sharp peak, and BENZ appeared in a symmetrical peak but after 16min as a retention time which is so tedious and time consuming. Using sodium acetate buffer resulted in sharp accurate peak for ALP, and BENZ eluted as a symmetrical peak after 9 min which is a reasonable time for drug analysis, and hence time consuming.

3.5.3. The mobile phase flow rate

The mobile phase was delivered at different flow rates (0.5, 0.6, 0.75, 1 and 1.5 $mLmin^{-1}$) where optimum separation with reasonable analysis time was obtained with a flow rate of 1 $mLmin^{-1}$.

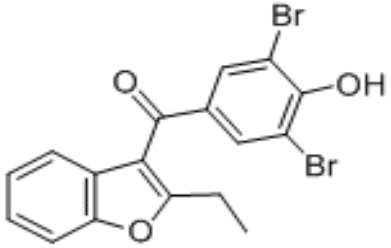
3.5.4. Wave length detection

Several wavelengths were tried such as 220, 230, 240, 250 and 260nm where the last one gives the highest detector response with acceptable noise to signal ratio for ALP and BENZ.



(1H-pyrazolo [3, 4-d] pyrimidin-4-ol)[1]

Fig 1. *Allopurinol (ALP) structure*



(3,5-dibromo-4-hydroxyphenyl)-(2-ethyl-3- benzofuranyl) methanone

Fig .2 *Benzbromarone (BENZ) structure*

After method optimization, the chromatographic separation was performed on Zorbax C_{18} column with a mobile phase consisting of acetonitrile: sod acetate buffer: triethylamine (50: 50: 0.5, by volume) at pH=4.5 adjusted with acetic acid delivered at 1 $mLmin^{-1}$and the detection of the separated peaks at 260 nm. The obtained chromatogram is shown in **Fig 7.**

3.5.5. Results

The developed Tlc-Densitometric and RP- HPLC methods have been successfully applied for determination of the binary mixture in Alloben® tablets, **Table 1**.

Moreover, the results obtained from the suggested methods were statistically compared to the published RP-HPLC one [11] (using F and student's t-test) with no significant difference among the proposed methods, **Table1.** Regarding both accuracy and precision.

Table 1. *Determination of the studied drugs in the laboratory prepared mixtures (L.P.) and pharmaceutical preparation by the proposed methods and statistical comparison with the reported RP-HPLC methods*

Parameters	TLC method		RP-HPLC method	
	ALP	BENZ	ALP	BENZ
L.P Mixtures[a]	98.71± 0.940	99.19± 0.938	98.68± 1.196	100.39± 1.285
Alloben® tabletsb (B.No.100251)	99.40± 1.447	98.66± 0.842	100.47± 0.954	98.46 ± 0.565
Standard addition[a]	99.72± 0.569	98.97± 0.519	97.96± 1.107	98.93± 0.543
Degree of freedom[c] F-test	2.454 (2.787)*	1.954 (2.450)*	0.496 (2.210)*	1.241 (2.357)*
Degree of freedom[c] Student's- test	4.214 (4.818)*	3.247 (4.224)*	2.097 (4.245)*	3.846 (3.971)*

4. METHODS VALIDATION

After satisfactory development of the method, it was subjected to method validation which was covering all criteria of ICH guidelines[14].The method was validated to demonstrate that it is suitable for its intended purpose by the standard test procedure to evaluate adequate validation characteristics.

5. LINEARITY

Beer's Lambert's law was obeyed in the concentration ranges of 0.3-3.00µgmL-1 for ALP and BENZ (for Tlc-Densitometric method) and in the range of 1-50, 3-50 µg mL-1for ALP and BENZ, respectively (for RP- HPLC method).The evaluation parameters like correlation coeffients, intercept and slope were calculated and presented in Table2.

6. ACCURACY

Accuracy was checked by applying the proposed methods for determination of different blind samples of pure ALP and BENZ. The concentrations were calculated from the corresponding regression equations and the results were presented in Table2.

Accuracy of the method was further assured by applying the standard addition technique on different pharmaceutical dosage forms where good recoveries were obtained.

7. PRECISION

7.1. Repeatability

Three concentrations of ALP and BENZ were analyzed three times intra-daily using the proposed methods. Good %RSD was obtained, confirming the repeatability of the method as shown in Table2.

7.2. Intermediate Precision

The previous procedure was repeated inter-daily on three different days for the analysis of the chosen concentrations. Acceptable %RSD was obtained and given in Table2.

Table 2. *Regression and analytical parameters of the proposed methods for determination of Allopurinol and Benzbromarone*

Parameters	TLCmethod		HPLC method	
	ALP	BENZ	ALP	BENZ
Calibration range	0.3-3µgmL^{-1}	0.3-3µgmL^{-1}	1-50µgmL^{-1}	3-50µgmL^{-1}
Slope	0.5693	0.2003	0.0147	0.0380
Intercept	0.1668	0.0478	0.0028	-0.0261
Correlation coefficient(r)	0.9999	0.9998	0.9999	0.9999
Accuracy	100.25	100.53	99.85	99.53
Precision				
Repeatability	1.174	0.935	0.470	0.859
Intermediate precision	0.662	1.354	1.190	0.755

8. SPECIFICITY

Specificity of the method was tested by how accurately and specifically the analytes of interest are determined in the presence of other components (e.g.: coformulated drugs, excipients and impurities, etc).This is evident from Tlc-Densitograms in **Figures 3-6** and HPLC-chromatogram in **Figure7.**The good percentages obtained by applying the proposed method on pharmaceutical dosage forms, **Table 1.**Also proved the specificity of the proposed methods. Fig .1 Allopurinol (ALP) structure

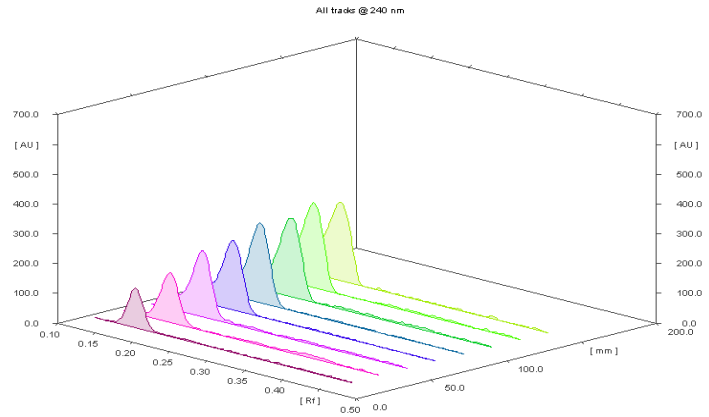


Figure 3. *TLC-Densitogram of Allopurinol in the concentration range of 0.3 - 3 µg band*$^{-1}$

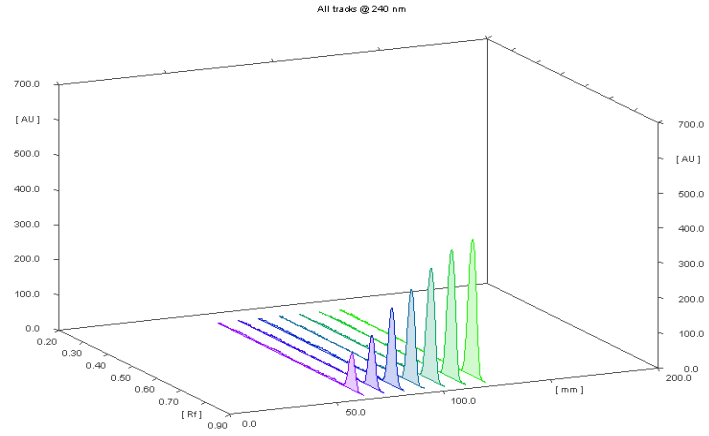


Figure 4. *TLC-Densitogram of Benzbromarone in the concentration range of 0.3 - 3 µg band*$^{-1}$

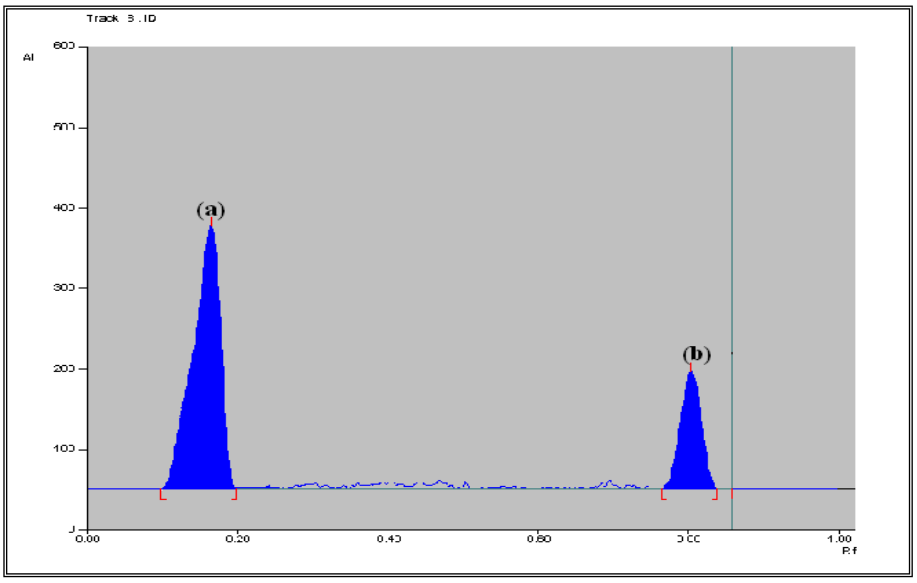


Figure 5. *Two dimensional TLC-Densitogram of (a) Allopurinol and (b) Benzbromarone mixture. Using chloroform: methanol (92:8, v/v) as a developing system and 240 nm as a scanning wavelength.*

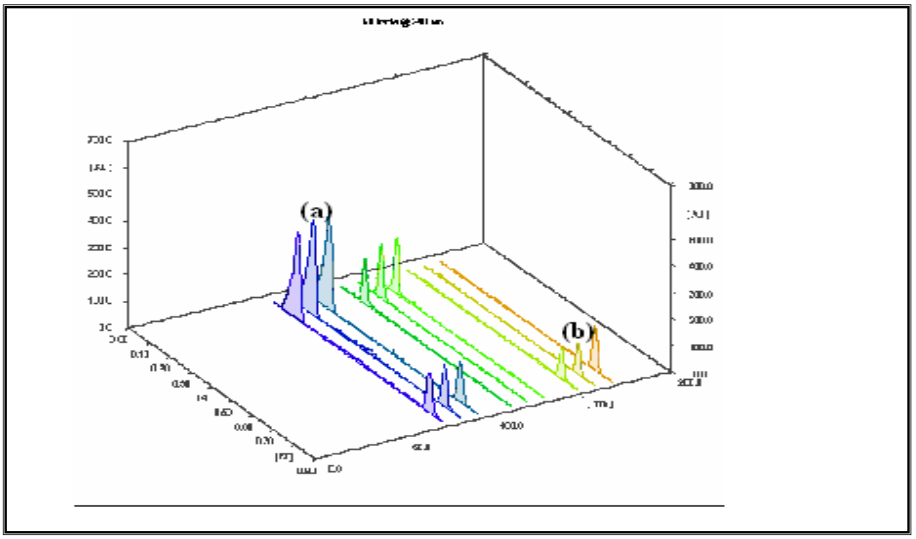


Figure 6. *Three dimensional TLC-Densitogram of (a) Allopurinol and (b) Benzbromarone mixture using chloroform: methanol (92:8, v/v) as a developing system and 240 nm as a scanning wavelength*

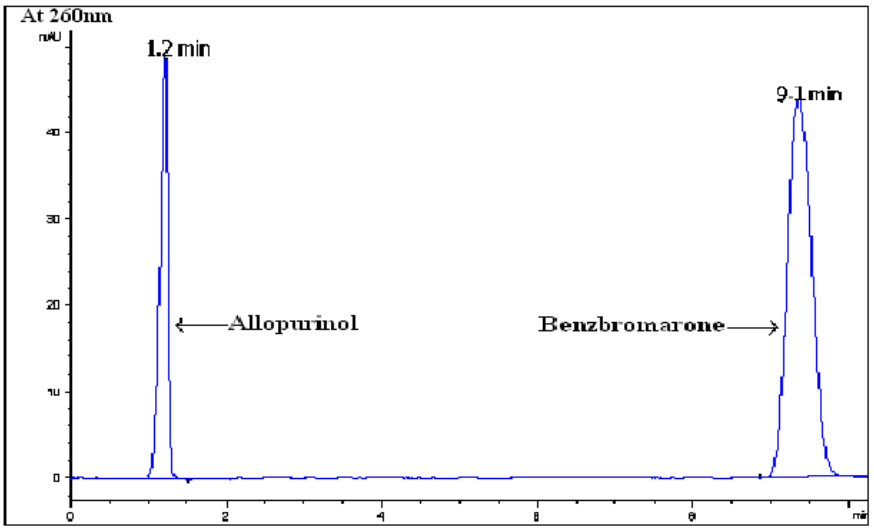


Figure 7. ***HPLC*** *chromatogram of 20 µg mL^{-1} of ALP and 10 µg mL^{-1} of BENZ using acetonitrile: sodium acetate buffer: TEA (50: 50: 0.5, by volume) pH=4.5 as a mobile phase at 260 nm.*

9. ROBUSTNESS

Tlc-Densitometric method: Robustness is the capacity of the method to remain un- changed with small changes in method parameters e.g.: changing saturation time ±5 min and changing the scanning wavelength ±1 nm. The low value of %RSD shows that the method is robust and that deliberate small changes in the studied factors did not lead to significant changes in R_f values, area or symmetry of the peaks.

RP-HPLC method: Small deliberate variations of the experimental conditions were applied in order to determine the effect on retention time, resolution and peak area. Changes in mobile-phase composition (±2%), flow rate (±0.2 $mLmin^{-1}$) or buffer pH (±0.1 pH unit) did not affect significantly the chromatographic method illustrating the robustness of the method.

10. SYSTEM SUITABILITY

An overall system suitability testing was done to determine if the operating system were performed properly. Parameters including resolution (Rs), peak symmetry and selectivity (α). Factors were calculated where good results were obtained and peak information were given in **Tables 3, 4.**

Table 3. *System suitability testing parameters of HPLC method. HETP [a] = height equivalent to theoretical plates (cm/ plate)*

Parameters	Obtained value (ALP)	Obtained value (BENZ)
Resolution (Rs)	4.3	
Selectivity factor (α)	1.26	
Tailing factor (T)	1	1.2
Capacity factor (k')	4.4	3.3
Number of theoretical plates (n)	25695	45143
HETP [a]	5.6×10^{-2} cm	3.8×10^{-3} cm

Table 4. *System suitability testing parameters of TLC-Densitometric method*

Parameters	Obtained value	
	ALP	**BENZ**
A Symmetry factor	1. 25	1.00
Resolution (Rs)	3	
Selectivity factor (α)	1.55	
Retardation factor	0.16	0.75

11. CONCLUSION

In the present work sensitive and selective Tlc-Densitometric and RP-HPLC methods for the determination of ALP and BENZ in their pure form, laboratory prepared mixtures and dosage form has been developed and validated. Firstly, the developed Tlc-Densitometric method is considered superior to the reported method in being more selective, sensitive, pH independent and can be used for determination of ALP and BENZ at single wave length compared to the reported one. It can be used for the chromatographic separation of the studied mixture using one developing system and scanning at single wave length, which lowers the analysis time and cost.

Secondly, the developed RP-HPLC method has the advantages in being time consuming and cost effective because it has less retention time and the peaks are more sharp and symmetric than the reported one. Thus, a result in signal-to-noise ratio is enhanced. Moreover, all the obtained results confirmed the applicability, accuracy and precision of these methods.

REFERENCES

[1] The British Pharmacopoeia, Her Majesty's.The stationary office, London(2009)

[2] The United States Pharmacopeia,30Ed.,National Formulary25, United States Pharmacopoeia convention Inc.(2007)

[3] H.Breithaupt, G.Goebel, Determination of allopurinol and oxipurinol in biological fluids by High- Performance Liquid Chromatography, *J.Chromatogr.B.*, (1981), 226, 237-242.

[4] F.Palmisano, E.Desimoni, P.G.Zambonin, Simultaneous determination of allopurinol, Oxipurinol and uric acid in body fluids, *J Chromatogr.*,(1984),14,205-306

[5] P. Fuchs, W.E. Haefeli, H.R. Ledermann, M. Wenk, Xanthine Oxidase inhibiting by allopurinol affects the reliability of urinary caffeine metabolic ratios as markers for N-acetyltransferase2 and CYP1A2 activities, *European J. Clin. Pharmacol.*, (1999), 54, 869-876.

[6] H.Breithaupt, Kinetics of Allopurinol after single intravenous and oral doses, *European J. Clin. Pharmacol.*, (1982), 22, 77-84.

[7] H.Yamanaka, K.Nishiokak, T.Suzuki, K.Kohno, Allopurinol metabolism in a patient with xanthine oxidase deficiency, *European J. Clin. Pharmacol*., (1983), 42(6):684-6.

[8] I. Walter-Sack, J. X. Varies, C. Kutschker, A. Ittensohn, Disposition and uric acid lowering effect of oxipurinol: comparison of different oxipurinol formulations and allopurinol in healthy individuals, *European J. Clin. Pharmacol. A. ,* (1995), 49, 215-220.

[9] M. Renling, Z. Honghaua, P. Sheban, Q. Yiqun, Determination of benzbromarone tablets by HPLC, *Zhongguo-Yiyao-Gongye-Zazhi*, (2002), 33(1), 30-31.

[10] Y. S. Fenxi, Pharmacokinetics of benzbromarone in normal chinese males, *Chia Nan Coll.Pharm Sci.*, (1999), 7(2), 107-114.

[11] M. Nebsen, M.A.K.EL-Rahman, M.Y.Salem, A.M.EL-Kosasy, and M.E.G.Bardicy, Simultaneous Determination of Allopurinol and Benzbromarone in presence of the active metabolite Oxipurinol, *Anal. Chem.* An Indian Journal, (2011), 10, 4.

[12] V. Nitsche, H. Mascher. Determination of benzbromarone in serum after combined therapy with allopurinol, Firma Gerot Pharmazeutika, Abt.*Biopharmakokinetik*.1981, 31(I)(3), 510-12.

[13] F. O. Muller, R. Schall, G. Groene woud, H. K. L. Hundt, J. C. Merwe, The effect of Benzbromarone on allopurinol/oxypurinol kinetics in patients with gout. *European J. Clin. Pharmacol*.1993, 44, 69-72.

[14] ICH, Q2 (R1) Validation of Analytical Procedures, 2005, Proceedings of the International Conference on Harmonization, Geneva.

[15] R. A. Sodhi, J. L. Chawla and R.T. Sane, Simultaneous determination of paracetamol, ibuprofen and chorzoxazone by HPLC, HPTLC and GC methods. *Indian Drugs.* 1996, 33,280-285.

[16] M. S. El-Masry, I. S. Blagbrough, M. G. Rowan, H. M. Saleh, A. Aboul Kheir, P. J. Rogers, Quantitative HPLC analysis of mebeverine, mesalazine, sulphasalazine and dispersible aspirin stored in a venalinic monitored dosage system with co-prescribed medicine, *J. Pharm. Biomed. Anal.*, 2011, 54,646-652.

[17] E. A. Abdelhamid, N. S. Abdelwahab.Validated chromatographic and spectrophotometric methods for analysis of some amoebicide drugs in their combined pharmaceutical preparation, *Pak. J. Pharm. Sci.*, 2013, 26,175-183.