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ADVANCED TECHNICAL AND MATHEMATICAL ASSESSMENT OF PEAK PURITY AND IDENTITY IN SALTING- OUT THIN LAYER CHROMATOGRAPHY FOR DETERMINATION OF SOME ORAL HYPOGLYCEMIC DRUGS

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ABSTRACT

Four advanced technical and mathematical methods for assessment of peak purity and identity in salting out thin layer chromatography (SOTLC) of some oral hypoglycemic drugs have been concluded. The concluded methods help in accurate assessment of coefficient of similarity between the spectra for each compound as a pure substance or exists in pharmaceutical preparations. The study extended to derive equations for calculating the overlapped peak area between two adjacent peaks when the resolution between 0.0 and 1.0 and helping in reducing measurement errors (manual and automatic integration of the integrators).

KEYWORDS: Peak purity and identity; integration; resolution; overlapped area; salting out thin layer chromatography; oral hypoglycemic drugs.

1. INTRODUCTION

Oral hypoglycemic drugs are used for treatment of diabetes mellitus type II.[1] The studied oral hypoglycemic drugs are; glibenclamide, glimepiride, gliclazide, pioglitazone HCl, metformin HCl and repaglinide, the chemical structures[2] are in **Figure 1**. Selectivity of an analytical procedure characterizes the extent to which given analytes can be determined simultaneously without interferences in presence of other components while specificity means that the one individual analyte can be undisturbedly measured in a real sample by a specific reagent, a particular sensor or a comparable measuring system. Moreover, the concepts of selectivity and specificity are used interchangeably and synonymously.[3] The specificity of a separation method can be evaluated by study of peak purity and identity test which used to demonstrate that an observed chromatographic peak is attributable to a single component.[4] Running a peak purity check prior to analytical quantitation helps to ensure accuracy and it is also a useful addition to routine quality control procedures, especially in the analysis of pharmaceuticals and food products for which contamination and quality of results are critical.[5] Under optimally defined conditions for separation and determination, a pure compound will produce a peak with spectra that have the same shape across the peak. In contrast interference fromnco-migrating analytes will produce composite spectra with various degree of spectral dissimilarity across the peak. This is the basis for peak purity detection and determination. One approach facilitates the assessment of spectral similarity is to view the spectrum as a vector that is a quantity has a magnitude expressed in length and direction. The length is proportional to analyte concentration while direction is proportional to the spectral shape.[6]

$$Cos(a,b) = \frac{\sum(a_i b_i)}{\sqrt{\sum(a_i)^2 \sum(b_i)^2}} \quad (1)$$

where a_i and b_i coordinates of vectors of a and b, while cosine of both spectra equals to correlation coefficient r.

$$r = \frac{\sum\left[(a_i - \bar{a})(b_i - \bar{b})\right]}{\sqrt{\sum(a_i - \bar{a})^2 \sum(b_i - \bar{b})^2}} \quad (2)$$

On the other hand sin and the Euclidean distance (e) between the tips of the two vectors are measures of spectral dissimilarity that can be expressed in the following equations;

$$Sin(a,b) = \sqrt{1 - \cos^2(a,b)} \quad (3)$$

$$e = \sqrt{2 - 2.\cos(a,b)} \quad (4)$$

Another mathematical expression of spectral similarity can be given by equation[7]

$$b_i = ma_i + x_i + n_i \quad (5)$$

Where b_i and a_i are the digitized absorbance at specific wavelength of two spectra to be compared, m is a multiplier and x_i is the spectral contribution of an unknown interferences and n_i is the noise. The unavailability of an exact mathematical solution to the spectral comparison is the reason for many alternative techniques, each of which uses a number of different matching procedures in assessing spectral similarity. However, none of the procedures produce an absolute answer. Salting out thin layer chromatography (SOTLC) is a reversed phase thin layer chromatography in which the used salt (ammonium sulfate) ionized in water and reversing the polar properties of silica gel sorbent to become nonpolar stationary phase, hence the nonpolar siloxane parts interact with the substances being separated, so more lipophilic drugs separated faster than the hydrophilic ones.[8] The main objective of this research was to study the different mathematical and technical methods for assessment of peak purity and identity in salting out thin layer chromatography of the cited oral hypoglycemic drugs to ensure the purity and identity of their samples in bulk and oral dosage forms. In addition in this study overlapped area between two adjacent peaks in resolution range 0.0–1.0 will be determined mathematically and confirmed practically by salting out TLC technique for analysis some oral hypoglycemic drugs.

2. Experimental

2.1. Equipment

A TLC scanner III (Muttenz, Switzerland) provided with linomat 5 sample automatic applicator (Muttenz, Switzerland) and 100 µL sample syringe (Hamilton, Bonaduz, Switzerland) was used. TLC tank (standard type) (27.0 cm width × 26.5 cm height × 7.0 cm diameter) (Sigma- Aldrich Co., USA) was used in this study.

2.2. Materials and reagents

All solvents and reagents used were of analytical grade. Glimepride (99.9%) was kindly supplied by Delta Pharma Co. (Cairo, Egypt). Glibenclamide (99.9%) was obtained from Hoechst AG (Frankfurt, Germany). Gliclazide (99.2%) was supplied by Servier. Repaglinide (≥98%) was obtained from Sigma Aldrich (USA). Pioglitazone HCl (≥98%) was supplied by Dr Reddy's, European Egyptian Pharmaceuticals. Metformin HCl (99.96%), methanol and ammonium sulfate were obtained from EI- Nasr Pharmaceutical Chemical Co. (Abu-Zaabal, Egypt). Thin-layer chromatography aluminum sheets precoated with 0.2 mm layers of silica gel 60 were obtained from Fluka (Silica gelmatrix H with fluorescent indicator 254 nm; Sigma Aldrich, Germany).

2.3. General assay procedure

The investigated compounds were dissolved in methanol at concentration of 250 µg/mL, corresponding to 1000 ng/band. About 50 mL of the mobile phase (ammonium sulfate: acetonitrile, 7:3 v/v) was poured into the TLC tank, which was lined with a thick filter paper to help the chamber saturation. Then, the tank was covered and pre-saturated with the mobile phase system for at least 30 min at room temperature (25 ± 2ºC) before use. The samples were spotted in the form of bands with 4 mm of band length using microliter syringe on silica gel 60 plates (20 cm width × 6 cm length with 0.2 mm thickness using Linomat 5. A constant application rate of 150 nL/s was used. The slit dimension was kept 3 × 0.45 mm, using TLC scanner III in reflectance–absorbance mode. Four microliters of the sample solution were spotted on the marked start edge of the TLC Plate 1 cm apart from the lower edge of the plate. The plates were then allowed to air-dry for about 5 min, then transferred to the TLC tank and developed with the specified mobile phase for 9 min (approximately 1 cm apart from the upper edge of the plate). After development, the plates were removed, air-dried for about 5 min and the separated bands were scanned at 237 nm using TLC scanner III. The obtained data were treated with win CATS software version 1.4.4.6337.

3. RESULTS AND DISCUSSION

In fact in thin layer chromatography the resolution of the separated peaks can be used as an indicator of overlay or interferences, as resolution (*Rs*) for pair of peaks can be calculated using equation[9]

$$Rs = \frac{Z_2 - Z_1}{0.5(w_1 + w_2)} \qquad (6)$$

where Z_1 and Z_2 are retardation factors of matched peaks (standard and sample or of adjacent peaks in a chromatogram),w_1 and w_2 are peaks widths at baseline of peak 1 and 2. According to literature for *Rs*=0.5, peaks still overlap each other by about 20%. At a resolution of 1, the peak profiles only overlap by 3%, of course, separation is required for reliable quantification, which corresponds to a resolution of *Rs* 2.5[10] this is only true for symmetrical peaks adhering to a Gaussian profile. The good saturation time of the mobile phase, good selection, adjustment of the mobile phase ratio, using automatic sample applicator at a constant application rate, adjustment of band length with specific speed of scan ensure the symmetry of the resultant peaks. The aforementioned facts were confirmed practically by our study as shown in **Figure 2** which showing the resolution of co-migrating peaks at various resolution values ranging from 0.19 up to 2.67 and scanning was carried out at co-absorptive wave length at λ237 nm using ammonium sulfate and acetonitrile in the ratio 7:3 (v/v) as a mobile phase. In this study, the following methods were concluded for assessment of peak purity and identity of the studied drugs.

3.1. Evaluation of peak purity and identity by calculation of correlation coefficient along scanned spectra

TLC scanner III is controlled with a personal computer and provided with winCATS software that will perform these calculations needed for peak purity and identity

test.

Peak purity and identity test procedure using winCATS software

The purity and identity test must be performed on a single plate for both authentic and sample in same concentration**.** Scanning of the bands in the specified wave length in the range of 200 to 400 nm. Adjusting the parameters of purity and identity test in winCATS by checking on purity and identity in dialog box, correlation limit adjusted to be 0.9970. After scanning, the results checked for matched spectra, the results either fail or pass, in our work all spectra passed at the selected limits.[11] During the purity test the spectrum taken at the first peak slope is correlated with the spectrum of the peak maximum, this correlation is identified as r (s,m) in winCATS, with s indicating peak start and m peak maximum. The correlation of the spectrum taken at the peak maximum with the one from the down slope or peak end named r (m,e), is used as a reference for the following statistical calculation. The null hypothesis "these spectra are identical" can in this case (purity) with two sided significance and an error probability of 1% only be rejected if the test value (Z) is greater than or equals to 2.576, Z is calculated as.

$$Z = \frac{[In\frac{1+r(s,m)}{1-r(s,m)} - In\frac{1+r(m,e)}{1-r(m,e)}}{2*\sqrt{\frac{2}{N-3}}} \quad (7)$$

The better the correlation of the start to maximum spectra, the narrower the range allowed for the correlation of the maximum to end gets. The spectra of dosage form and reference standard were compared. The results of the peak purity and identity test are shown in **Table 1**, r (s,m) and r (m,e) for authentic repaglinide (*auth*) were 0.9997 and 0.9995 ;respectively while for repaglinide tablet sample solution (*samp*) were 0.9998 and 0.9995; respectively. The similarity factor (*S*) can be calculated using the following equation.

$$S = \frac{r(s,m).r(m,e)_{samp}}{r(s,m).r(m,e)_{auth}} \quad (8)$$

The similarity factor *S* was 0.9999. The closeness of peak purity values to 1 indicated that the analyte chromatographic peak was only attributed to a single compound. Peaks with purity values below 0.950 have a high probability of being impure. Peaks with values between 0.950 and 0.990 can be suspected of being impure. Peaks with values above 0.990 are probably pure, subjected to the limitations of the technique.

3.2. Peak purity evaluation regarding peak area calculations

The winCATS software calculating the peak area at maximum (apex) position that is corresponding to R_f value and above the base line when scanning at maximum wavelength (λ_{max}). In determination of repaglinide R_f value 0.50±0.01 for both standard and tablet sample solution, also both solutions have the same three maxima; 212,248 and 293 nm. Scanning was at λ_{max} 293 nm and dividing peak area of tablet sample by standard peak area using 1.0 µg/band of both authentic and sample to calculate the recovery which was 100.11% ± 1.36. The similarity factor (*S*) was 1.001 which was calculated as the ratio of the peak area of tablet sample (PA_{samp}) and the authentic solutions (PA_{auth}). The allowed limits 1.00 ± 0.02 (1.02, 0.98) between two standards.[12] The similarity factor (*S*) calculated using equation.

$$S = \frac{PA_{samp}}{PA_{auth}} \quad (9)$$

3.3. Peak purity evaluation by comparison of matched peaks R_f values

Peak purity and identity can be evaluated by comparison of the R_f values of the pure substance and R_f values of tablet. Since R_f value is characteristic for any given compound, it can provide corroborative evidence to the identity of a compound. In winCATS[11], the R_f value can be calculated for each peak at start, maximum and end of the scanned band position after scanning at λ_{max} (293 nm). So the comparison of R_f values for spectra adds additional evidence of purity and identity of a substance. In this study, start, maximum and end R_f values were; 0.42, 0.50 and 0.58 ±0.01 for authentic repaglinide and were; 0.42, 0.50 and 0.56 ±0.01 for sample of repaglinide in tablets **(Figure 3**). There is no significant difference in the R_f values at different positions between the compared peaks, identical and pure substances have same R_f values ±0.03-0.05 according to the preferred R_f range.[13]

3.4. Evaluation of the purity and identity by overlay of the compared spectra and peaks

The spectra of the authentic and sample tablets of repaglinide when scanned in the range of 200-400 nm gave a spectrum with three λ maxima; 212,248 and 293 nm as shown in **Figure 4**. Due to that λ_{max} is a characteristic value for each compound, thus the similarity in wavelength maxima indicating the identity of the compared spectra. The same when authentic and sample scanned at specific λ_{max} 293 nm gave peaks that can be superimposed for additional confirmation of purity and identity. In winCATS software after spectra and peaks are acquired, they are overlaid for visual evaluation in three dimensional (3D) view and setting up horizontal and vertical angle views at zero as in **Figure 4** and **Figure 5**, however; no conclusion can be drawn concerning the kind and level of impurity.[14] A good overlay, where peak shape and retention or migration time match, indicates a pure peak while a poor overlay indicates an impure peak.

This study extended to determine the overlapped area of incompletely separated peaks which represents a challenge in instrumental analysis when the resolution between two peaks in the range 0.0-1.0 and by knowing

R_f values of matched peaks and start or end position of the overlapped peaks. The study was based on the determination of the overlapped peak area by applying triangular method. The calculated area according to the equation[15];

$$A = 0.5HB \qquad (10)$$

where *H* is the height at perpendicular position to the base, *B* is the width of the peak (R_f values). The overlapped area calculated theoretically using a theoretical model (**Figure 6)** and practically when resolution 0.0, 0.25, 0.40, 0.70 and 1.0 between glibenclamide (regarded as the main peak), repaglinide, glimepiride, pioglitazone HCL and gliclazide to test the ability of application. Then the area was correlated with the resolution using R_f values in calculations and the linear least square equation was concluded. The relationship was direct inversely because we take the down slop half of the main peak (glibenclamide) that was overlapped with other drugs peaks (r = -0.943,y=a+bx, t_f =0.08,t_{th} =6.39, F_f=1.12,F_{th}=2.31). The theoretical and practical overlapped area results were compared, there was no significant difference between the theoretical and practical results according to *F* and *t* tests. The peak area then integrated by applying triangulation method and the general formula was developed that relating overlapped area and resolution. This method introduces a new trend in analytical methods for determination of impurities and interferences and can be applied as an integration method in the analytical integrators to overcome the errors of measuring the area of the overlapped peaks by manual and automatic base line correction that used by many integrators. In addition we hope this work participate to resolving of overlapping peaks that are characterized with homogeneity and symmetricity. By study the relationship between the overlapping area and the resolutions of co-migrating peaks (1 regarded as the main peak, 2, 3, 4; glibenclamide, repaglinide, glimepiride and pioglitazone HCl respectively) with R_f values in the range (0.20-0.36) and the calculated resolution was in the range of 0.25 to 0.70, we suggested the following integration formula applying basic integration formula[16] that relating R_f values with area of overlapped peaks in TLC using overlay method and concerning **Figure 2** and **Figure 6**.

$$OA_1 = 2\int_{0.34}^{0.36}(a+bx)dR_f \qquad (11)$$

$$OA_2 = 2\int_{0.32}^{0.36}(a+bx)dR_f - 2\int_{0.34}^{0.36}(a+bx)dRf \qquad (12)$$

$$OA_3 = 2\int_{0.29}^{0.36}(a+bx)dR_f - 2\int_{0.32}^{0.36}(a+bx)dRf + 2\int_{0.34}^{0.36}(a+bx)dRf \qquad (13)$$

$$OA_{Tot} = OA_1 + OA_2 + AO_3 = 2\int_{0.29}^{0.36}(a+bx)dRf + 2\int_{0.34}^{0.36}(a+bx)dRf \qquad (14)$$

$$OA_{Tot} = 2\left[ax + 0.5bx^2\right]_{0.29}^{0.36} + 2\left[ax + 0.5bx^2\right]_{0.34}^{0.36} \qquad (15)$$

The peak area here equals to whole area of the peak and can be represented as OA_{Tot}. Due to the direct relationship between R_f values and resolution the former equations can be expressed as.

$$OA_1 = 2\int_{0.70}^{1.0}(a+bx)dR_s \qquad (16)$$

$$OA_2 = 2\int_{0.40}^{1.0}(a+bx)dR_s - 2\int_{0.70}^{1.0}(a+bx)dR_s \qquad (17)$$

$$OA_3 = 2\int_{0.25}^{1.0}(a+bx)dR_s - 2\int_{0.40}^{1.0}(a+bx)dR_s + 2\int_{0.70}^{1.0}(a+bx)dR_s \qquad (18)$$

$$OA_{Tot} = OA_1 + OA_2 + AO_3 = 2\int_{0.25}^{1.0}(a+bx)dR_s + 2\int_{0.70}^{1.0}(a+bx)dR_s \qquad (19)$$

$$OA_{Tot} = 2\left[ax + 0.5bx^2\right]_{0.25}^{1.0} + 2\left[ax + 0.5bx^2\right]_{0.70}^{1.0} \qquad (20)$$

The concluded formula characterized by simplicity and deal deeply with the interference and overlapping of two adjacent peaks.

Table 1: The results of peak purity and identity test of repaglinide determination using the proposed method and obtained by win CATS Software.

	Standard substance				Dosage form (tablet)			
	100 ng/band	200 ng/band	500 ng/band	1000 ng/band	100 ng/band	200 ng/band	500 ng/band	1000 ng/band
r (s,m) [a]	0.9998	0.9995	0.9996	0.9999	0.9997	0.9997	0.9998	0.9999
r (m,e) [a]	0.9996	0.9998	0.9991	0.9996	0.9995	0.9999	0.9991	0.9995

[a] Average of three replicates .

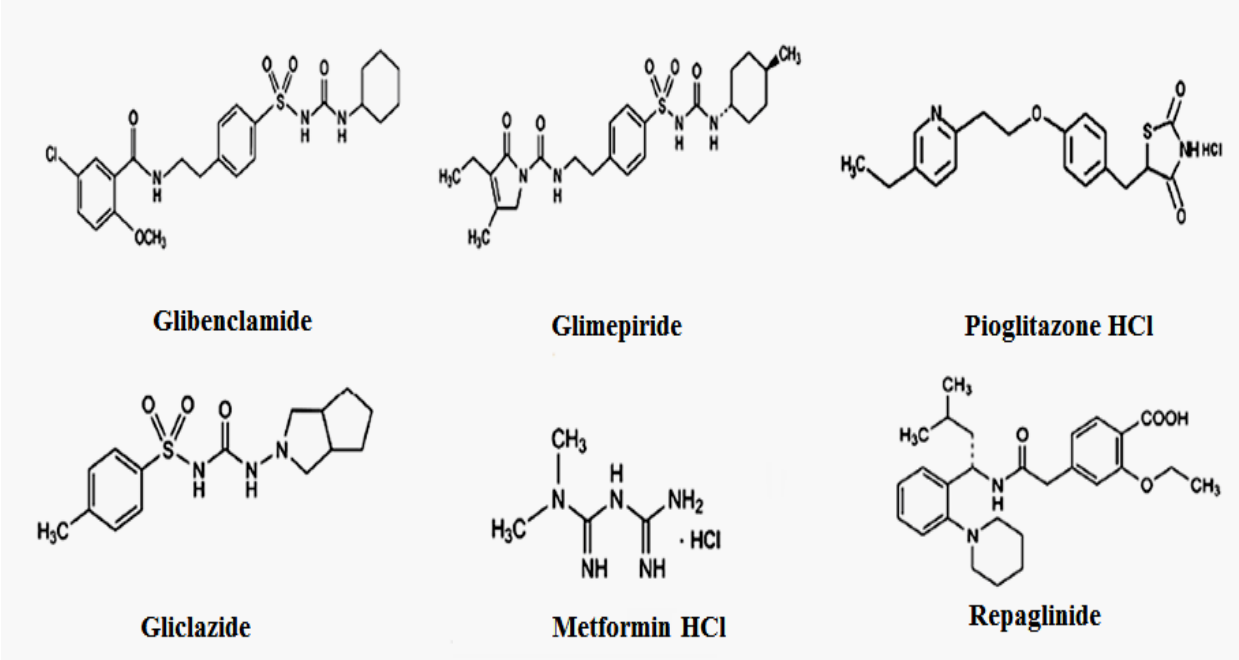

Figure 1: Chemical structure of the investigated drugs.

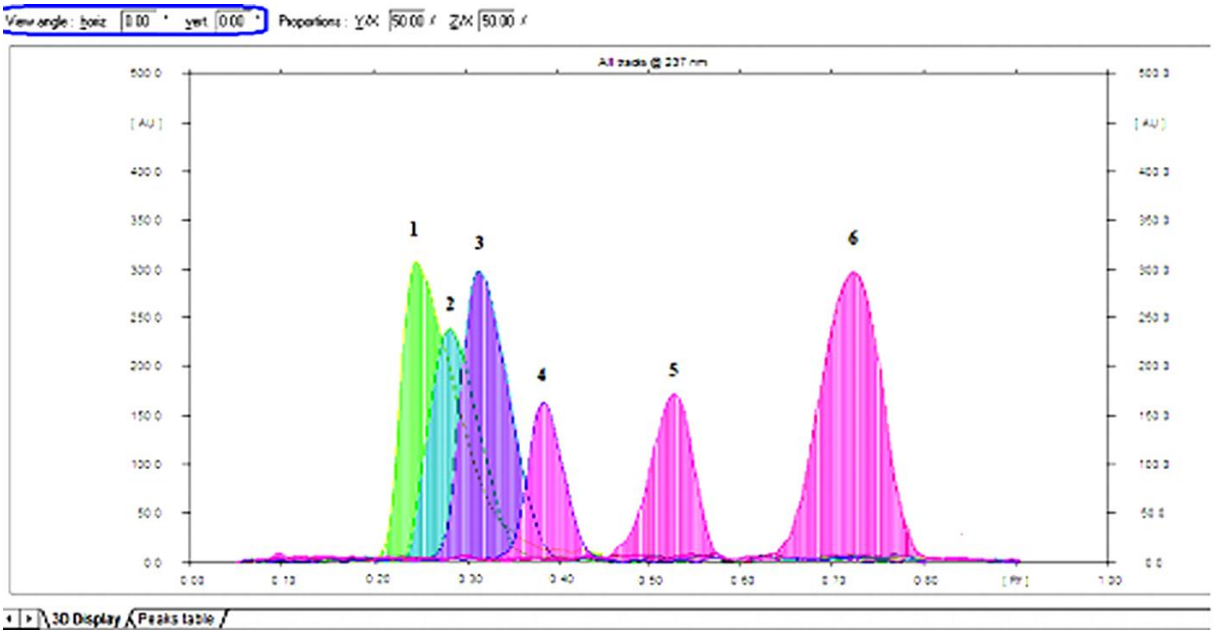

Figure 2: 3D graph for identification and determination of (1) glibenclamide (as main peak), (2) repaglinide, (3)glimepiride , (4) pioglitazone HCl, (5) gliclazide and (6) metformin HCl, using 0.025M ammonium sulfate: acetonitrile 7:3 (v/v) as a mobile phase and scanning at λ 237nm by TLC scanner III.

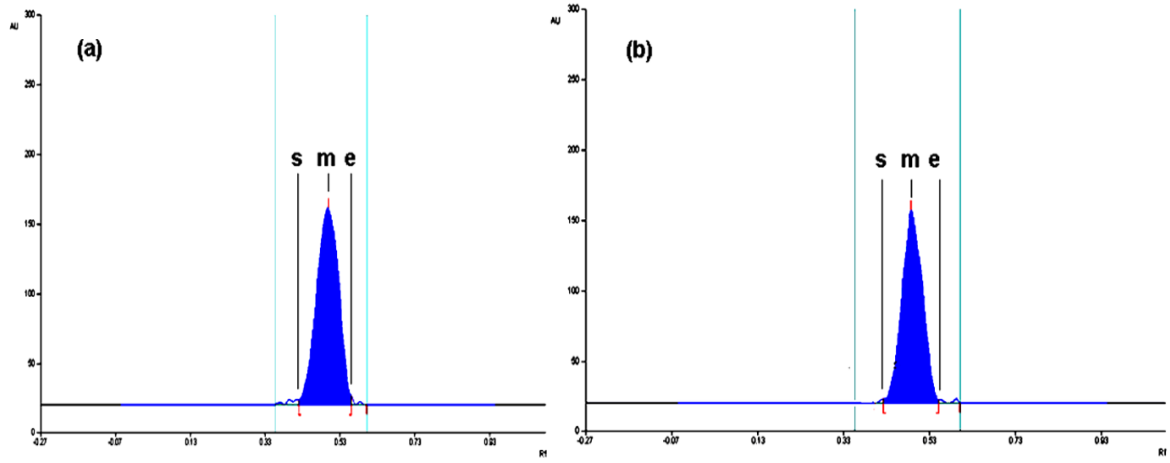

Figure 3: Densitograms matching (a) authentic repaglinide and (b) tablet solution containing 500 ng/band of repaglinide, s,m,e ; are start ,maximum and end position of scanned bands at λ_{max} 293 nm using TLC scanner III.

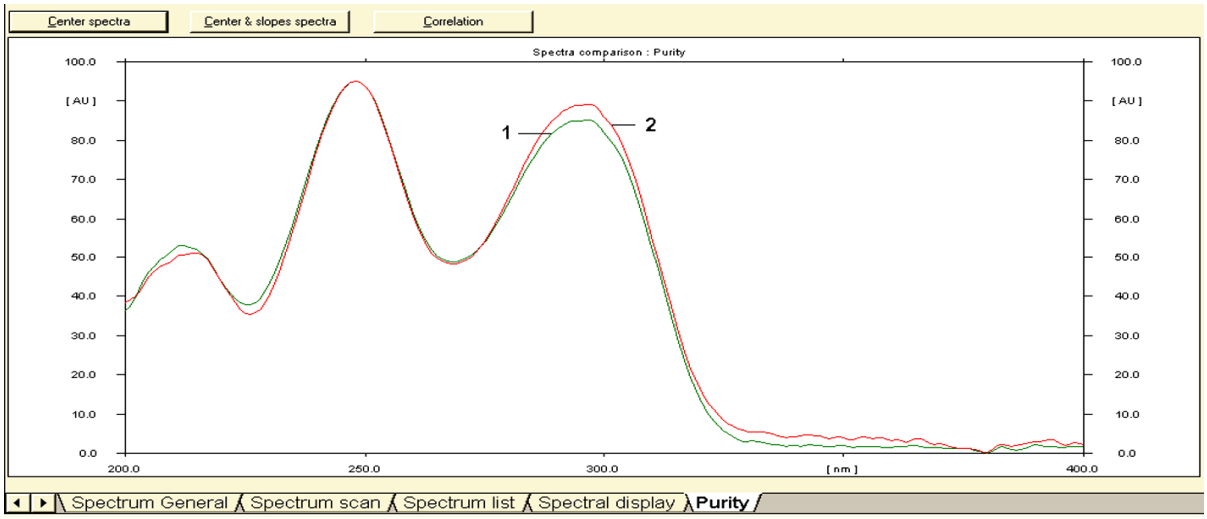

Figure 4: Spectra comparison of (1) standard solution containing 1000 ng/band of repaglinide and (2) sample solution 1000 ng/band of repaglinide selected from the spectrum list obtained by winCATS software.

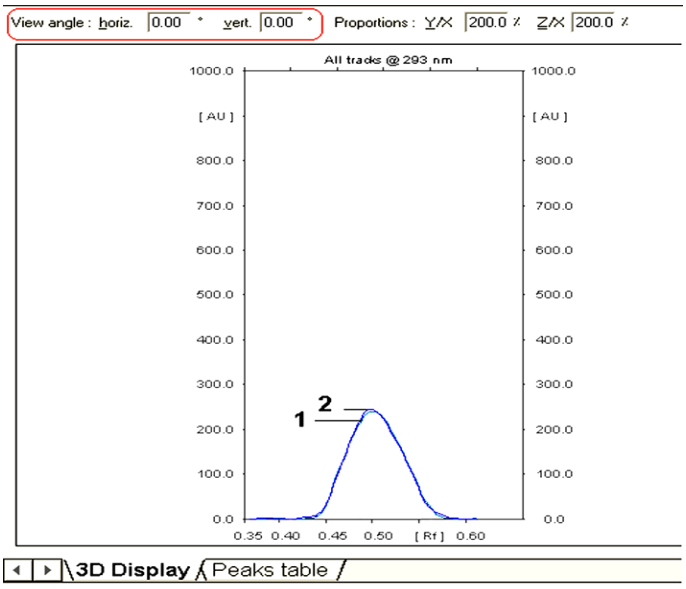

Figure 5: 3D graph of compared and overlaid peaks; (1) Authentic repaglinide solution and (2) tablet sample solution each contains 1000 ng/band.

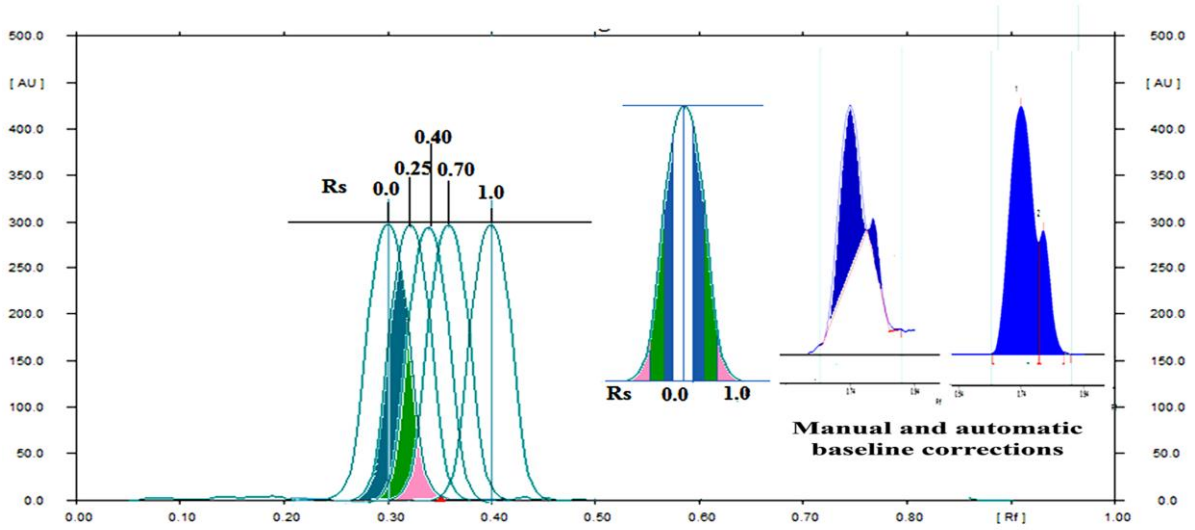

Figure 6: The theoretical model for determination of peak area (related to R_f, resolution) manual and automatic baseline correction.

4. CONCLUSION

This research deals with more accurate, reliable mathematical and technical evaluation methods for assessment of peak purity and identity. The concluded methods included peak purity and identity test using r (correlation coffecient) of standard and sample spectra. Other methods included comparasion of R_f values, peak area, overlay of studied peaks. This work is a contribution to resolve the problem of overlapping peaks (when *Rs* between 0.0 and 1.0) that are characterized with homogeneity and symmetricity. The derived equations for calculating the overlapped peak area between two peaks may help in reducing analytical errors (manual and automatic integration) of integrators and recommended to be applied in instrumental analysis.

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REFERENCES

1. Harvey RA, Champe PC, Lippincott's Illustrated Reviews: Pharmacology, Lippincott Williams & Wilkins, Philadelphia, 2009; 291-292.
2. O'Neil M J, Heckelman PE, Koch CB, Roman KJ, Kenny CM, D'Arecca MR,"The Merck index", Merck & Co.,Inc. Whitehouse Station, NJ, USA, 2006; 765–766, 773,1025, 1284, 1402.
3. Danzer K, Analytical chemistry, Theoretical and metrological fundamentals, Analytical performance characteristics, Springer-Verlag Berlin Heidelberg, 2007; 189-190.
4. Ahuja S, Dong MW, Handbook of pharmaceutical analysis by HPLC, Validation of HPLC methods in pharmaceutical analysis, Elsevier academic press, Elsevier Inc, 2005; 200-201.
5. Cazes J (ed), Papadoyannis IN, Gika HG, Encyclopedia of Chromatography, Peak purity determination with a diode array detector, Marcel Dekker, 2005; 1.
6. Jilliard JA, Ritter C. Use of simulated liquid chromatography- diode array detection data for the definition of a guide curve in peak purity assessment by spectral comparasion, J.Chromatogr. A, 1997; 786: 1-11.
7. Malikin G, Application note, Assessing HPLC peak purity with shimadzu photodiode detector. SPD M10A vp.
8. Mohamed AI, Mohamed FA, Ahmed SA, Aboraia AS, Mohamed YA. Salting-out thin-layer chromatography and computational analysis of some oral hypoglycemic drugs, Biomed Chromatogr, 2014; 28: 1156-1162.
9. Sherma J, Fried B (ed), Kowalska T, Kaczmarski K, Prus W, Handbook of Thin- Layer Chromatography, 3 rd edn., Revised and Expanded, Theory and mechanism of thin-layer Chromatography, Marcel Dekker, Inc., New York, 2003; 55.
10. Spangenberg B, Poole CF, Weins C, Quantitative Thin-Layer Chromatography; Theoretical basis of thin layer chromatography (TLC), Springer Heidelberg Dordrecht London New York, 2011; 37.
11. winCATS software, Switzerland (1999-2008) version 1.4.4.6337.
12. Sharma N, Rao S, Reddy AM. A novel and rapid validated stability-indicating UPLC method of related substances for dorzolamide hydrochloride and timolol maleate in ophthalmic dosage form, Journal of Chromatographic Science, 2012; 50: 745-755.
13. Dhont GH. The RF value as a constant in thin-layer chromatography, Journal of Chromatography A, 1980; 202: 15-20.

14. Stahl M, Peak purity analysis in HPLC and CE using diode-array technology, Agilent Technologies, 2003; Publication number 5988–8647EN.
15. Smith RM (ed), Dyson N, Chromatographic integration methods, 2nd edn., Measurements and models, Dyson Instruments Ltd., UK, Royal Society of chemistry, Thomas Graham House, Science Park, Milton Road, Cambridge CB4 4WF, UK, 1998; 28.
16. Barnes M, Introduction to Integration, Part 2: The definite integral, Mathematics Learning Centre, University of Sydney, 1999; 12.