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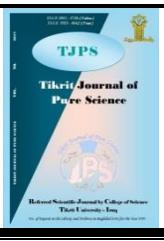

Ultra violet spectrophotometric analysis of Paracetamol, Diclofenac and Tramadol drugs in mixture

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ABSTRACT

The aim of this research was develop and validate an analytical method by Uv spectrophotometric technique for quantitative determination of paracetamol (PAR), diclofenac or voltarine (DIC) and tramadol or tramal (TRA) in tablet dosage form, paracetamol analysis is based on the absorbance maxima were found to be at 243 nm when dissolved in 0.1N H_2SO_4 as a sample and in 0.1N NaOH solution as a blank,. Quantitative of PAR in sample is achieved by standard addition methods and three ways calculations were used to estimate the amount in the tablet, the expected content per tablet were equal to (365.60, 361.984, 358.415 mg) and the results were acceptable when compared with original quantity in tablet(350 mg). The method was compared with standard curved method, showed that it is obeyed Beer – lambert law in the concentration range of 0.1–1 µg/ml for standard addition and standard curve methods, with a correlation coefficient of 0.9980 and o.9944. The limit of detection (LOD) for PAR was 0.036 µg/ml while limit of quantitation (LOQ) 0.119 µg/ml, the recovery of three procedure A, B, C of standard addition and standard curve were (104.40, 103.42, 102.40 and 92.995 %) for PAR ,it was found that the results obtained from the standard addition method were better than the result obtained from the standard curve method.

The amount of (DIC) and (TRA) drug in tablet sample calculated depending on the absorbance (A) at 273 nm to give the value 47.44 mg and 47.6 mg per tablet are acceptable when compared with the value of the original quantity in tablet (50mg) and the recovery of the method was found to be (95.2 and 96.0 %) respectively, the principle of the method based on the (A) of mixture at this λ is a total A of the two drugs, which owns different intensity at this λ at different percentages and that apply to the sample and standard for this drugs. Finally this can applied successfully for routine analysis.

Introduction

Paracetamol (PAR) (acetaminophen, N-acetyl-p-aminophenol) an analgesic and an antipyretic drug, PAR (4-hydroxylacetanilide) is used in the treatment of pain and inflammation, It is commonly used for the relief of headaches, and is a major ingredient in numerous cold and flu remedie[1,2].

It is frequently prescribed in amixture with other related drugs such as diclofanec and tramadol[3].

Diclofenac sodium (DIC) is an aryl propionic acid derivative belongs to non-steroidal anti-inflammatory drugs (NSAIDs), which have analgesics, antipyretics and anti-inflammatory activities, Tramadol hydrochloride (TRA) (trans-2- [(dimethyl-amino) methyl] -1-(methoxypheny) cyclohexanol, centrally acting opioid agonist provide analgesic effect by blocking µ-receptors[4,5], the combination medication always used to relieve symptoms caused by the common cold, flue, allergies or other breathing illnesses, one of these compensation is Sudafed[6]. Different analytical methods used for analysis of these of combination drugs, method employed TLC plates precoted with silica gel, the method was

validated for precision and accuracy[7,8]. HPLC –MS method was validated for analysis pseudoephedrine at ng /ml concentration for used in support toxicology studies[9], the HPLC method was successfully applied for routine analysis of these compounds in different cough and cold pharmaceutical preparation [10]. GC-MS was used of identification of PAR and TRA in pharmaceutical compound [11], Quantitation spectrophotometric determination of PAR with oxidizing agent for its determination [12,13].

Experimental

Instrumentation

A Shimadzu UV- Visible spectrophotometer model 1800 (Japan) with 1 cm matched quartz cells were used and a Sartorius digital Balance.

Chemicals and agents; (PAR) standard was provided by(sammraa drugs industrial) SDI, (DIC) and (TRA) tablets 50mg were obtained from market Sudafed tablets containing PAR (350 mg), DIC (50 mg) and TRA (50mg) from (brawn India). were used throughout the experiment.

The chemicals are of analytical grade H_2SO_4, NaOH, ethanol, are obtained from different companies.

Preparation of solvent; 1-dissolve 4g of NaOH in water to prepared 1000ml of 0.1N solution.

2- dilution of 2.77 ml concentration H_2SO_4 with dis-water to prepare 1000 ml of 0.1N solution.

3- Ten mg of PAR, DIC and TRA were accurately weighed and transferred to 100 ml volumetric flask. 25 ml of ethanol was added and sonicated for 10 minutes, and then volume was completed to the mark with dis- water.

Determination of wavelength at maximum absorbance; The standard solutions of PAR, (DIC) and (TRA) were once diluted with 0.1N H_2SO_4 and again with 0.1N NaOH, this solution was scanned in the range 200-400 nm. The wavelengths of maximum absorbance of PAR, DIC and TRA were found to be at 243, 272 and 269 nm in acidic and 258, 275, 270 nm in basic mediums as shows in figure 1 and 2.

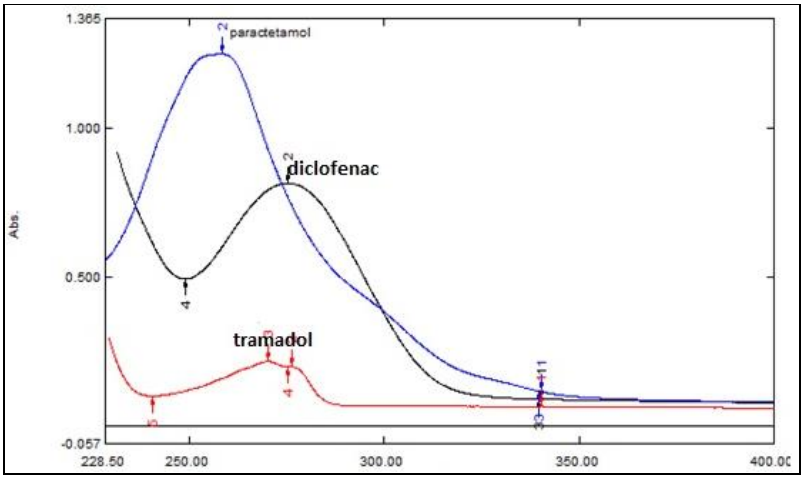

Fig. 1: Spectrum of PAR, DIC and TRA in acid solution

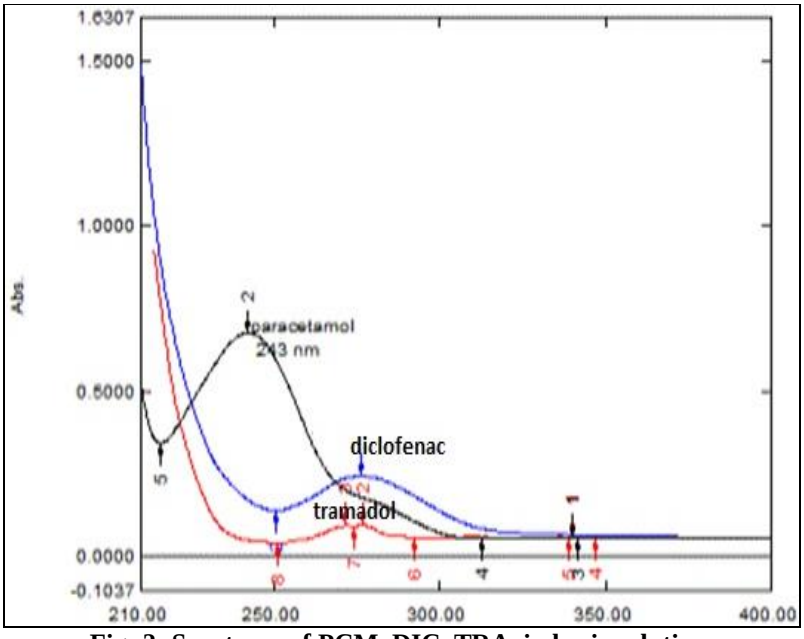

Fig.-2: Spectrum of PCM, DIC, TRA, in basic solution.

Determination of PAR by standard addition method (SAM); There are two common methods to perform standard addition method

1- We begin with an unknown solution and measure the analytical signal. Then, we add a small volume of concentrated standard and measure the signal again. We add several smaller volumes of standard to the constant volume of sample and measure the signal after each addition.

Preparation of standard and sample;

1- Ten tablets of Sudafed were weighed and crushed in fine powder (9700 mg).

2- 100 mg of Powder equivalent to 36.08 mg of PCM was accurately weighed and transferred to 100 ml volumetric flask. 25 ml of ethanol was added and sonicated for 10 minutes.

3- Insoluble excipients were separated by filtration using whatman filter paper no. 41. and then volume was completed to the mark with ethanol.

4- One ml of the filtrate was diluted with100ml of 0.1N H_2SO_4 to get final concentration of 0.36% mg (3.6 µg / ml) of PAR as sample.

5- One ml of the filtrate was diluted with 100 ml of 0.1 N NaOH as a reference.

6- 40 mg of PAR standard were accurately weighed and transferred in a 100 ml volumetric flask, 25 ml of ethanol were added and sonicated for 10 minutes and then volume was made up to 100 ml with ethanol.

7- Different volumes (0 ml, 1.0ml, 1.25ml, 1.5ml,1.750ml and 2.0 ml) equal to (0, 4, 5, 6, 7 and 8 µg/ml of standard) were added to six volumetric flask contain 1 ml of sample and diluted to 100 ml with 0.1N H_2SO_4.

Calculations

1- Reading were taken at 243 nm of sample solution with and without standard addition against the reference solution prepared by diluting the sample with 0.1N NaOH (blank) to give the following absorbance value; 0.341, 0.703, 0.793, 0.864, 0.972 and 1.082.

2- Absorbance of sample without standard addition (0ml of standard) = 0.341, (A1).

3- Absorbance of 1ml sample mixed with 1ml of standard = 0.703. (A2)

4- Absorbance of standard (absorbance difference) = 0.703- 0.341= 0.362, (A2-A1).

5- From the data above calculate the amount and % of PAR stated content in sample of Sudafed tablet by three equations as bellow was calculated;

- The equation; C sample =A sa /A st ×C st
- Amount expected in tablet powder analyzed equal to37.679 mg /100 mg,
- The amount of PAR per tablet =365.6

A- The equation = $[x]_i / [x]_f + [S]_f = Ax/A(s+x)$[14] , when the[x]i is the initial concentration of unknown sample $[x]_f$, $[S]_f$ are the final concentration of sample and standard, A $(_{s+x)}$ and Ax are the absorbance of sample with and without standard addition respectively.

to apply this equation and calculate the [x]i, by the following;

- To one ml of sample add 98 ml of 0.1N H_2SO_4 to become the final volume of sample 99 ml, and added one ml of standard to obtain 100 mL of the final volume of standard.
- [x] = 0.0037318 ×10000 = 37.318 mg PAR in 100mg powder.
- Amount of PAR in tablet =361.984

B- Standard addition method**;**

- Plot a calibration curve of absorbance vs concentration of standards and the amount of drug was calculated by the equation, y= 0.0912x + 0.3368.Fig-3.
- When y = 0, the x (C) = 0.3368/0.0912 = 3.6929 µg/ml =36.929mg per 100mg powder .
- Amount of PAR in tablet = 358.211mg

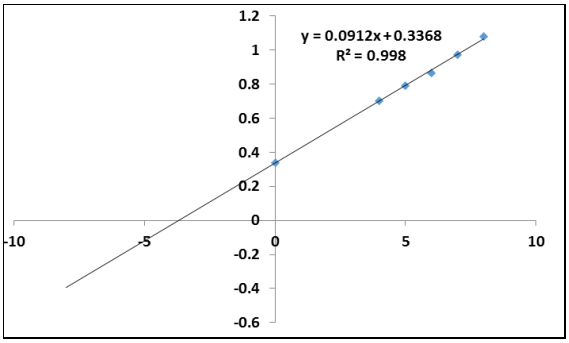

Fig. 3 : curve of PAR by standard addition method

Determination of paracetamol by standard calibration curve (SCM);

Preparation of standard solution;

1- Standard solution of PAR was prepared by dissolving 10 mg in 100 ml of 0.1N H_2SO_4 as stock solution.

2- Working standard prepared by serial dilution of stock solution with 0.1 N H_2SO_4 to obtain concentration range of 1. 2, 4, 6, 8 and 1.0, µg/ml.

Preparation of standard curve

1- Absorbance was measured at 243 nm against the sample dissolved in 0.1N NaOH as a blank.

2- Values of absorbance were plotted against concentrations and their linearity range was determined fig-4.

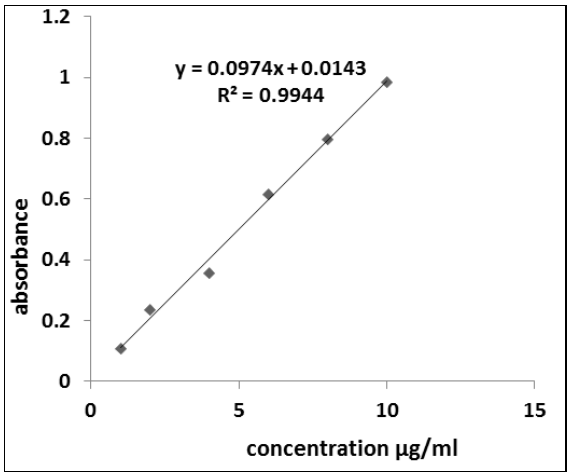

Fig. 4 : paracetamol standard curve

Calculation

Use the equation; y = (0.0974x+0.0143) to determine the concentration of PAR in the solution and use this to calculate the amount of PAR in the tablet.

According to the equation,

1- 0.341= 0.09736x + 0.0143. then x(C) = 3.354 µg/ml = 33.542mg in 100 mg sample.

2- The amount of PAR in tablet = 325.358 mg.

Determination of Diclofenac (DIC) and Tramadol (TRA);

Preparation of standard and sample;

1. Five tablets of each DIC and TRA were crushed in to a fine powder.
2. 10 and 25.7 mg of powder tablet equivalent to 2.5 mg of DIC and TRA were accurately weighted and transferred each one alone in 100ml volumetric flask.
3. A mixture of (10 and 25.7) of DIC and TRA powder tablet were accurately weighed and transferred in 100 ml volumetric flask.
4. 48.5 mg of Sudafed sample powder equivalent to 2.5 mg of DIC and TRA was accurately weighed and transferred in 100 ml volumetric flask.
5. add 20 ml of 0.1N NaOH to the three flasks and sonicated for 10 minutes to dissolve the powder.
6. Insoluble excipients were separated by filtration using whatman filter paper 41.
7. The volume of each flask were completed with 0.1N NaOH to mark.
8. Alone 25 ml from each flask transferred in 50 ml volumetric flask and completely to the mark to give 1.25% mg.

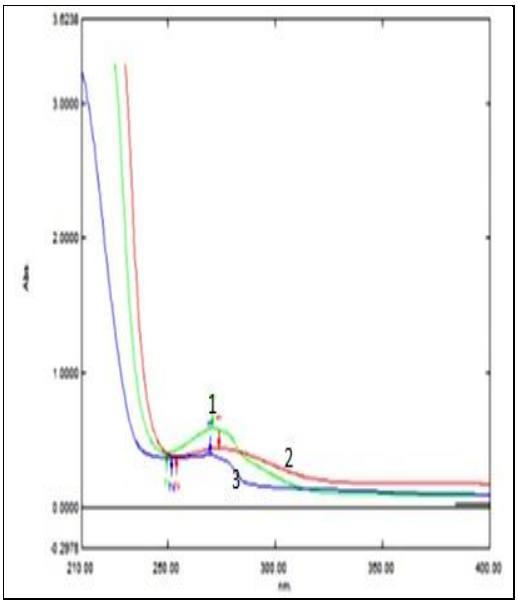

Fig.5. Spectrum of diclofenac (red), tramadol (blue) and the mixture (green)

Calculation

1- Absorbance of DIC, TRA and mixture were measured in 0.1N NaOH at 273 nm wavelength as average of 275 nm of DIC and 270 nm of TRA).

2- Absorbance of DIC, TRA and the mixture equal to 0.600, 0.204 and 0.831.

3- The A percentage (%) of DIC and TRA in mixture were calculated by 0.60 / 0.831×100 = 72.2% and 0.204 / 0.831 × 100 = 24.6% respectively.

4- According to the lambert law, A = A(1%1cm) × C × L, then, A(1%1cm), (the absorbance of 1g/100ml in 1cm cell). of DIC and TRA were calculated as bellow.

5- The dilution factor (df) =50/25 = 2

6- Concentration of DIC = (0.572 / 480) ×2 = 0.00238 g or 2.38mg in 48.5 mg of sample.

7- Concentration of TRA = (0.195/161.6) ×2 = 0.0024 g or 2.4 mg in 48.5 mg of sample.

8- Amount of DIC and TRA equal to 47.6mg and 48mg per tablet respectively.

Result and discussion

The standard addition method (SAM) was very efficient for correcting the matrix effect and providing an overall evaluation of the effect.

Calibration curve by standard calibration curve(SCM) and standard addition curve (SAM) methods (Figs- 3 and 4) was plotted between absorbance and concentration.

Use signal for unknown to find analyte in each tablet (Table 1),

Table (1). Absorbance A, Conc, %, and mg per tablet of paracetamol in Sudafed.

no	procedure	found content mg per 100mg	Ratio %	found content mg per tablet	Rec %
1	Standard addition A	37.76	104.9	365.6	104.4
2	Standard addition B	37.318	103.661	361.984	103.424
3	Standard addition C	36.929	102.638	358.211	102.346
4	Calibration curve	33.542	93.210	325.358	92.959

in SAM the concentration of the unknown solution was determined by dividing the intercept value by the slope of the sample when the y = 0.

In SAM method, the known quantities of analyte were added to the unknown (total) and from the increase in absorbance, we conclude how much analyte was in the original unknown by used three calculating procedures, the first one(A) was used when the absorbance of standard equal to (A total– A sample) to give 365.6 mg per tablet.

By second procedure (B) consider a standard addition in which sample with unknown initial concentration of analyte [x]i gives a signal Ax. Then a known concentration of standard [S] is added to an aliquot of the sample to give a signal (AS), then amount of PAR in sample equal to 361.98 mg per tablet. The last calculation procedure is the standard addition curve to give the amount of PAR in sample equal to 358.211mg per tablet (table- 1). In usual practice an accurate result is the one which matches very nearly with true value of a measured amount, compared the result show that the concentrations of the PAR were calculated from their corresponding regression equation is more accurate than other.

Quantification of samples were performed using calibration curves prepared by standards solution at a series of concentrations) was linear in the concentration ranges of (0.1-1 mg %) as described in (Fig 3), calibration curve was used to find out the concentration of the unknown analytic concentrations in samples by using the equation of straight line as in (table- 1) to give 325.358 mg of PAR per tablet.

Finally, were able to Compare and contrast the determinations of the unknown sample composition by external standard curve and standard addition method, the results obtained with standard addition method were better than that obtained with standard calibration method and most convenient to determine the amount in mixture.

An accurate determination of DIC and TRA compounds in tablet sample was obtained by the A = A(1%1cm) CL method, the concentrations of the drugs were calculated from their equation. the absorbance should be directly proportionate to the concentration of the sample at the same λ.

The DIC and TRA absorb at each other λ of maximum absorbance and A of mixture at this λ is combined of the tow drugs (table 2),

Table (2) A, A%, (A1%1cm) of DIC and TRA drugs standard

no	drugs	absorbance	Absorb %	A1% 1cm
1	DIC	0.600	72.2	480
2	TRA	0.204	24.6	161.6
3	mixture	0.831	---	665

they have different intensity but the λ remains at 271-275 nm solution (Fig 5), the absorbance was measured at 27 3nm to give 47.6 mg and 48 mg per tablet respectively (table 3).

Table (3) A, expected content and % of DIC and TRA in sample

no	Drug	absorb of mixture	Taken (mg)	absorb of each drug	found content mg in 48.5 mg sample	found content mg in tablet	Rec %	SD	Relative error%
1	DIC 50mg	**0.792**	**2.5**	**0.571**	**2.38**	**47.6**	**95.2**	**0.0361**	**4.8**
2	TRA 50mg			**0.195**	**2.4**	**48**	**96**	**0.3530**	**4**

Validation

Validation should address the performance of the analytical procedure under conditions of routine used and to prove that the measurement conditions and the equation used for the final result calculation include all the interferences which affect the final result, the regression equations of PAR in pharmaceutical analysis showed a good linearity over the range of (0.1% to 0.8 µg/ml) of standard add to the sample of ASM and 0.1 -1 µg/ml of SCM (n = 6), the correlation coefficients for the calibration curves were all approaching 0.9980 of ASM and 0. 9944 of SCM indicating a good linearity.

Six replicate samples of the same volume (1ml) of PAR were analyzed by methods, (Table 5), summarizes the values obtained for the main parameters, from the standard deviation (S) it can concluded that 68 % of the results of the analysis lies within the rang 99 ± 0.0109 of SAM and 99.9 ± 0.0214 of SCM, the aim in an analysis is to make SD as small a percentage of the value of true as possible.

The comparison is normally done with regard to the 'error'; Absolute error(AR) 0.87 of ASM and 2.525% of SCM are the difference between the experimental value and the true value, relative Error (RE) was lower than 3.00 % (2.411) indicating good accuracy of the ASM and 6.998% of SCM was over than ASM or 3%, indicating lower accuracy table(4).

Table (4) Relative and absolute error, % for procedure

no	procedures	Relative error %	Absolute error %
1	**Standard addition A**	**4.908**	**1.767**
2	**Standard addition B**	**3.288**	**1.184**
3	**Standard addition C**	**2.411**	**0.87**
4	**Calibration curve**	**6.998**	**2.525**

The Precision that was determined as ± RSD, was lower than 1.584% of SAM indicating good precision and 6.40% of SCM indicating low precision than ASM.

The critical value of (t- table) at the 99% confidence level for (5) Freedom degree is 3.36 of ASM, which are less than t table value, that means the methods have a good accuracy and 6.658 of SCM at 99.9

confidence level for (5) Freedom degree indicating the method have low accuracy than the other.

the results are summarized in Table 5.

The results show that acceptable relative standard deviations lower than 10 % were obtained for most of the pharmaceutical compound of two methods and describes the dispersion of the measurements around the mean value and describes also the precision of the measurements.

LOD and LOQ were found to be 0.036 and 0.119 µg/ ml of SAM and for SCM 0.0661 and 0.2205 µg / ml respectively, that indicating a good sensitivity of ASM than SCM.

The precision of many measurement methods is affected by the matrix of the test sample as well as the level of the characteristic. For these methods, comparison of the precision is best done on identical test samples.

The amount of DIC and TRA per tablet were calculated by equation A=A (1% 1cm) CL, and the percentage of relative error and absolute error were calculated equal to (4.8 % , 5.2 %) and (0.12%0.13%) respectively.

Table (5) Validation parameters results of paracetamol of two method

no	1	2	3	4	5	7	8
methods	$\bar{x}$	**SD**	**RSD%**	**LOD**	**LOQ**	**t**	**F**
SAM	**0.688**	**0.0109**	**1.584**	**0.036**	**0.119**	**3.36 at 99%**	**0.26**
SCM	**0.3343**	**0.0214**	**6.40**	**0.0661**	**0.2205**	**6.658 at 99.9%**	**3.855**

Conclusion

The proposed spectrophotometric method with applied standard addition procedure for calculating was found to be simple sensitive accurate and precise for determination of PAR in mixture. The developed method was validated by testing its linearity, accuracy, precision, limits of detection and quantitation and specificity. DIC and TRA determination method is good enough to determination the amount of active ingredient in combined dosage form. The excipient is usually present in the solution do not interfere in this method.

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تحليل ادوية الباراسيتامول, الدايكلوفينيك والترامادول في خليطهما باستخدام طيف الاشعة فوق البنفسجية

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الملخص

هدف هذا البحث الى تطوير طريقة بسيطة وذات حساسية تعتمد على طيف الاشعة فوق البنفسجية لتقدير الباراسيتامول, الداي كلوفنك (فولتارين) والترامادول (ترامال) في المستحظرات الصيدلانية. تقدير الباراسيتامول يعتمد على قيمة المتصاصية عند الطول الموجيnm 243 عند اذابته في محلول حامض الكبريتيك كنموذج مجهول وثم اذابته في محلول هيدروكسيد الصوديوم كبلانك, قدرت كمية الباراسيتامول في النموذج الدوائي بتطبيق طريقة الإضافة القياسية وباستحدام ثلاثة طرق حسابية وكانت النتائج (365.60, 361.984, 358.415 mg) في القرص الدوائي الواحد والتي أظهرت مقبولية عند مقارنتها مع طريقة المنحني القياسي, تبين من خلال النتائج ان الطريقتان تتوافق مع قانون بير– لامبرت بمدى من التراكيز (0.1–1 µg/ml)، وكانت قيم معامل الارتباط 0.9944 ,o.9980 وحدود الكشف والكم 0.036µg/ml و0.119), وقيم الاستردادية الناتجة من تطبيق الطرق الحسابية الثلاثة كانت % 104 ,103 ,102, تمت مقارنة النتائج مع النتائج المستحصلة من تطبيق طريقة المعايرة القياسية (325.483 ملغم) واظهرت النتائج ان طريقة الإضافة القياسية بحساباتها الثلاثة اكثر ملائمة لتقدير الباراسيتامول عند وجوده في خليط من الادوية.

تم تقدير كمية عقاري الدايكلوفنك والترامادول في نفس الخليط واعتمادا على ان هذه الادوية تظهر امتصاصية بنسب وشدة مختلفة عند نفس الطول الموجي (273nm) لتعطي قيم كيمة لهذه الادوية (47.44, 47.60) ملغم والتي كانت مقبولة مقارنتا بكميتهما الفعلية في النموذج الدوائي(50 ملغم), إضافة الى ذلك فان قيم الاستردادية كانت تساوي 96.0 ,95.2 %على التوالي لكل منهما.

ان المبدأ الأساسي لهذه الطريقة يتضمن ان الامتصاصية لخليط هذين الدوائين هو عبارة عن مجموع امتصاصيتهما عند نفس الطول الموجي وبنسب مختلفة والتي تطبق على النموذج المجهول والقياسي.

واخيرا يمكن تطبيق هذه الطرق بنجاح في العمل الروتيني لتحليل تلك المواد .