DETERMINATION OF MICRO AMOUNT OF PARACETAMOL IN PHARMACEUTICAL PREPARATIONS BY MOLECULAR SPECTROPHOTOMETRIC METHOD

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Abstract

A simple and sensitive spectrophotometric method has been established for the determination of paracetamol. The method is based on the reaction of paracetamol with iron(III) sulfate. The produced iron(II) reacts with potassium hexacyanoferrate (III) forming Prussian blue colored product with a maximum absorption at 710 nm. Linearity was in the range 0.1 – 7.0 µg mL^{-1} paracetamol [2.5 – 175.0 µg / 25 mL] with a limit of detection (signal : noise = 3) of 0.038 µg mL^{-1}, a molar absorptive of 3.477×10^4 L mol^{-1} cm^{-1}, a Sandell sensitivity of 4.348 ng cm^{-2}, a relative error of less than ± 1.5% and a relative standard deviation of 0.745 – 1.002% depending on the paracetamol concentration. The proposed method was statistically applied to the determination of paracetamol in pharmaceutical preparations. The proposed method has been statistically evaluated with British Pharmacopeia method and no statistical difference between methods was found at the 95% confidence level.

Keywords: Paracetamol; Spectrophotometric; Hexacyanoferrate(III); Pharmaceutical preparations.

Introduction

Paracetamol (acetaminophen) is a chemical name of (N-acetyl-p-aminophenol). It is important and extensively used antipyretic – analgesic drug [1].

Several methods have been reported for determination of paracetamol in pharmaceutical preparations. These include spectrophotometry [2 – 8], polarography [9], mic-ellar electrokinetic chromatography [10], flow injection-spectrofluorimetric [11], flow injection Fourier-transform infrared spectrometry [12], flow injection-spectrophotometry [13, 14], first derivative spectrofluorimetry [15], square-wave voltametry [16], high performance thin-layer chromatography [17] and reversed-phase capillary electrochromatography [18].

Iron(III) salt and potassium hexacyanoferrate(III) have been used to determination of folic acid in tablets [19].

Most of spectrophotometric methods needs, either pre-hydrolysis step [20] or temperature and pH controls [8]. Therefore, development of a simple and sensitive spectrophotometric method seems to be desirable. The proposed method was based on the oxidation reaction of paracetamol with ferric sulfate in the presence of potassium hexacyanoferrate (III) forming Prussian blue colored product. This method offers the advantages of simplicity, no need for extraction or heating, in addition to higher sensitivity than some of the existing spectrophotometric method.

Experimental Apparatus

A Shimadzu UV–Visible 260 digital double-beam recording spectrophotometer (Kyoto, Japan) was used for all spectral and absorbance measurements with matched 1 cm quartz cells.

Reagents

All chemicals used were of analytical reagents grade. Paracetamol standard material was provided from the State Company for Drug Industries and Medical Appliances (SDI), Samarra – Iraq.

1- Paracetamol stock standard solution (1000 µg mL^{-1}) was prepared by dissolving 0.1000 g of pure paracetamol in 5 mL of ethanol with diluting to the marked with distilled water in 100 mL

volumetric flask. Working standard solutions were prepared by suitable dilution of the stock standard solution.

2- Ferric sulfate solution (0.005 M) was prepared by dissolving 0.2810 g of ferric sulfate in distilled water and diluting to the marked in 100 mL volumetric flask.
3- Potassium hexacyanoferrate (III) solution (0.005 M) was prepared by dissolving 0.1647 g in distilled water and diluting to the marked in 100 mL volumetric flask.
4- HCl concentrated solution (11.64 M).

Pharmaceutical preparations of paracetamol

Pharmaceutical preparations were obtained from commercial sources.

1- Paracetol tablets (SDI, Iraq): 500 mg paracetamol for each tablet.
2- Panadol extra tablets (Smithkline Beecham, Ireland): 500 mg paracetamol, 65 mg caffeine for each tablet.
3- Panatol tablets (Global Pharma, UAE): 500 mg paracetamol for each tablet.
4- Kanagesic tablets (Kanawati Medical Products, Syria): 450 mg paracetamol and 35 mg orphe-nadrine citrate for each tablet.
5- Emidol tablets (Global Pharma, UAE): 500 mg paracetamol for each tablet .
6- Hayamol injections (Ibn-Hayyan Pharmaceutical HOMS, Syria): 375 mg paracetamol for each injection (5 mL).
7- Panatol suppositories (Delta for Medicaments, Syria): 125 mg paracetamol for each suppository.

Analytical procedure

Aliquot of standard paracetamol (2.5 – 175.0 µg) was transferred into 25 mL calibrated flasks. To each flask, 3 mL of ferric sulfate solution (0.005 M), shake well and followed by 2 mL of potassium hexacyanoferrate(III) solution (0.005 M), then 3 mL of concentrated HCl (11.64 M) was added. The contents were diluted to the mark with distilled water and mixed; after 30 min, the absorbance value at λ_{max} = 710 nm was measured against a reagent blank and a calibration graph was constructed.

Procedure for the assay of pharmaceutical preparations

1- Tablets solution (1000 µg mL^{-1})

The average tablet weight was calculated from the contents of 20 tablets that had been finely powdered and weighed . A portion of this powder, equivalent to 100 mg of paracetamol, was accurately weighed. The sample weight was shaken with 5 mL of ethanol and diluted with distilled water in a 100 mL volumetric flask. The solution was filtered into a 100 mL volumetric flask.

2- Injections solution (1000 µg mL^{-1})

The contents of fine injections were mixed. An aliquot corresponding to 100 mg of paracetamol (1.3 mL) was shaken with 5 ml of ethanol and diluted to 100 mL with distilled water in a volumetric flask.

3- Suppositories solution (1000 µg mL^{-1})

One suppository was weighed and dissolved in 5 mL ethanol then complete the volume to 100 mL with hot distilled water in a volumetric flask. The solution was filtered in to a 100 mL volumetric flask.

Further appropriate solution of pharmaceutical preparations were made by using distilled water. Two different concentrations of each solution of pharmaceutical preparation were analyzed in five replicate by recommended procedure.

Results and Discussion

The proposed spectrophotometric method for the determination of paracetamol is based on the oxidation reaction of paracetamol with iron (III) and subsequent chelation with hexycayanoferrate (III) to form a Prussian blue colored product (21).

Spectral characteristics

A blue complex is formed when paracetamol was allowed to react with iron (III) salts in the presence of hexacyanoferrate (III) with maximum absorption at 710 nm as shown in Fig. (1). The reagent blank has practically negligible absorbance at this wavelength.

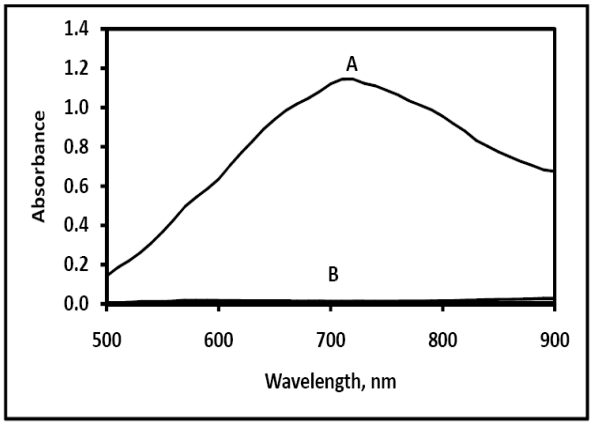
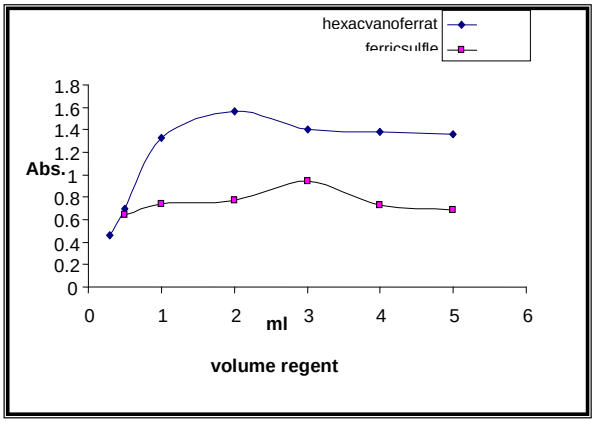


Fig. (1): Absorption spectra of A (5 µg mL^{-1}) of paracetamol treated as described under procedure and measured against a reagent blank and B the reagent blank measured against distilled water.

Optimum conditions for complex formation

In order to establish the optimum conditions necessary for a rapid and quantitative formation of the colored product with maximum stability and sensitivity, the investigators measured the absorbance of a series of solutions by varying one and fixing the other parameters at 710 nm.

It was found that a 0.005 M solution of ferric sulfate in the range (0.5 – 5.0 mL) and 0.005 M solution of pota-ssium hexacyanoferrate(III) in the range (0.3 – 5.0 mL) were necessary to achieve the maximum color intensity of the product. The color intensity decreased below the lower limit and above the upper limit.Fig.(2) shows that 3 mL of ferricsul fate and 2mL of hexacyano ferrate (III) (0.005M) where enough to obtain the maximum absorbance. Therefore, 3 mL of ferric sulfate and 2 mL of hexacyano-ferrate(III) were recommended for all measurements.



Fig(2) : optimum conditions for determination of paracetamol

Dilution of the blue product with different solvents like water, acetic acid, nitric acid, sulfuric acid, phosphoric acid and hydrochloric acid were tested. In water medium, the blue color product precipitates out. Results showed that concentrated hydrochloric acid (11.64 M) gives clear blue color with maximum intensity and stability compared to acetic acid, nitric acid, sulfuric acid and phosphoric acid. It was found as shown in Fig.(3) that 3 mL of concentrated hydrochloric acid in the range of (0.1 – 7.0 mL) was necessary to get clear, sensitive and stable blue color. Therefore, a 3 mL of concentrated hydro-chloric acid was recommended for all subsequent measurements.

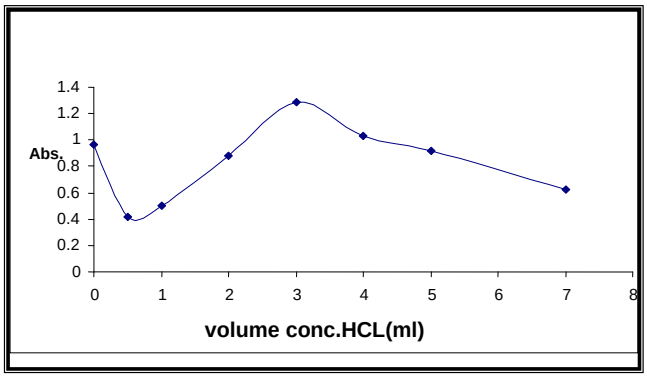


Fig.(3): best volume of concentrated hydrochloric asid for determination of paracetamol.

Reaction equation

Paracetamol reduces iron(III) salts in aqueous medium to form iron(II) salts, which subsequently chelate with hexacyanoferrate(III) to form Prussian blue colored product (21).

Paracetamol (reductant)+ $Fe^{3+} \rightarrow Fe^{2+}$ + oxidized product

$Fe^{2+} + K_3[Fe(CN)_6] \rightarrow KFe[Fe(CN)_6] + 2K^+$

Prussian blue

(Potassium ferric ferrocyanide)

Effect of temperature on colored product

The reaction between paracetamol and iron (III) salts in the presence of hexacyanoferrate (III) was found to be instantaneous. However, the reaction is complete within 10 min at room temperature (25° C), but 30 min was sufficient to get maximum intensity and stability color after the addition of concentrated hydrochloric acid and

distilled water to the final solution. The effect of temperature on the colored product was studied at 5, 25 and 45° C and the result obtained Fig.(4) indicated that the color was stable for at least 120 min at 25° C and was used in the analytical procedure.

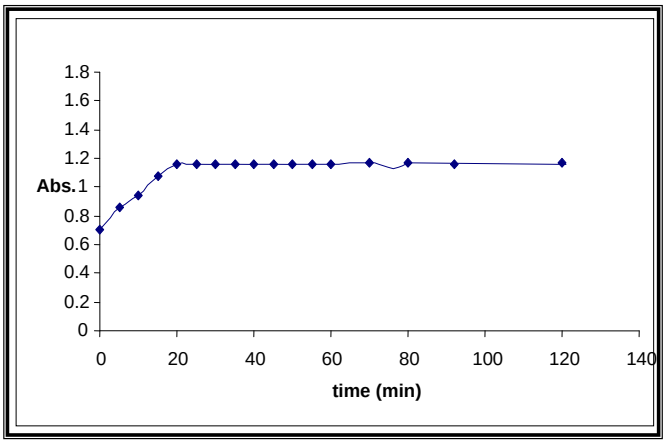


Fig.(4): effect of time (min) on absorbance for determination of paracetamol.

Analytical parameters

Under the optimum conditions described above, calibration graph for paracetamol Fig .(5) was const-ructed by plotting absorbance values as a function of the analyte concentration. The calibration graph for the individual determination was linear in the range of 0.1 – 7.0 µg mL^{-1} for paracetamol. The analytical and regression parameters of proposed method are summarized in Table (1).

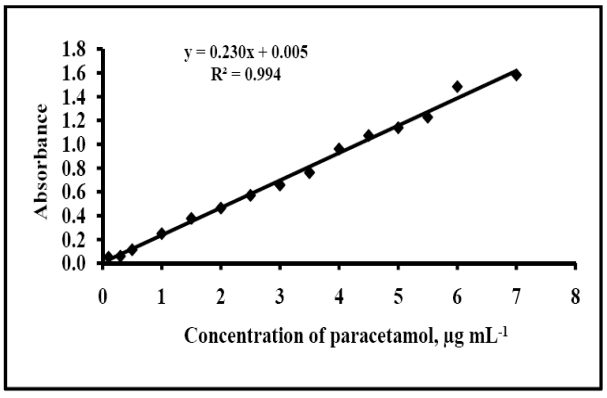


Fig. (5): Calibration graph for paracetamol.

Sensitivity parameters such as molar absorptivity (ε) and Sandell sensitivity values and the limits of detection (LOD) and quantification (LOQ) calculated (22) are also compiled in Table (1) and demonstrate the high sensitivity of the method.

Table (1)
Analytical and regression parameters of spectrophotometric method.

Parameter	Value
λ_{max}, nm	710
Linear reange, µg mL^{-1}	0.1 – 7.0
Molar absorptivity, L mol^{-1} cm^{-1}	3.477×10^{4}
Sandell sensitivity, ng cm^{-2}	4.348
LOD, µg mL^{-1}	0.038
LOQ, µg mL^{-1}	0.078
R^2 , correlation of detremination	0.9940
Intercept	0.005
Slope, mL μg^{-1}	0.230

Accuracy and precision

To evaluate the accuracy and precision of the method, a series independent standard of pure paracetamol was used at two different concentrations were determined. The results shown in Table (2), indicate that a good precision and accuracy could be obtained by the proposed method.

Table (2)
Accuracy and precision of the proposed method.

Concentration of paracetamol µg mL^{-1}		RE, %	Recovery, %	RSD, %
Taken	Found*			
2.000	1.993	- 0.355	99.645	0.745
5.000	4.934	- 1.320	98.680	1.002

RE is relative error, RSD is relative standard deviation.

****Mean value of five determinations.***

Interferences

To test the efficiency and selectivity of the proposed analytical method to pharmaceutical preparations, a systematic study under the optimum experimental conditions was made for the effect of additives and excipients such as starch, talc, lactose, magnesium stearate and polyvinylpirrolidone (PVP) that are usually present in a dosage forms. The criterion of interference was an error of not more than ± 1 % in the absorbance. In this study, a wide range of concentration was used in which the determination of the 2 μg mL^{-1} level of a drug was performed. Experimental showed that there was no interference from additives or excipients for the examined method up to 10-fold excess as shown in Table (3).

Table (3)
Determination of 2 μg mL^{-1} of paracetamol in the presence of excipients.

Excipient, 20 μg mL^{-1}	Concentration of paracetamol, μg mL^{-1} Found*	RE %	Recovery %	RSD %
Starch	1.9988	-0.060	99.940	0.314
Talc	1.9975	-0.125	99.875	0.531
Lactose	2.0070	+0.350	100.35	0.766
mg stearate	1.9863	-0.685	99.315	0.213
PVP	2.0170	+0.850	100.85	0.661

Mean value of five determinations.

Analytical applications

To evaluate the analytical applicability of the proposed method, it was successfully applied to determine of paracetamol in pharmaceutical preparations. The results are gives in Table (4), the recoveries are close to 100% which indicates that there is no serious interference in the determination of paracetamol in such samples.

Table (4)
Determination of paracetamol in pharmaceutical preparations.

Pharmaceutical preparation	Concentration of paracetamol, μg mL^{-1} Taken	Found*	RE, %	Recovery, %	RSD, %
Paracetol tablets	2.0000 5.0000	1.9750 4.9840	-1.250 -0.320	98.750 99.680	0.731 0.347
Panadol extra tablets	2.0000 5.0000	1.9900 4.9660	-0.500 -0.680	99.500 99.320	1.225 0.678
Panatol tablets	2.0000 5.0000	1.9800 4.9770	-1.000 -0.460	99.000 99.540	0.534 0.495
Emidol tablets	2.0000 5.0000	1.9580 4.9500	-0.750 -1.000	99.250 99.000	0.817 1.090
Kanagesic tablets	2.0000 5.0000	2.0180 5.0660	+0.900 +1.320	100.900 101.320	1.130 1.730
Hayamol injections	2.0000 5.0000	1.9930 4.9690	-0.345 -0.620	99.660 99.380	0.979 0.837
Panatul suppositories	2.0000 5.0000	2.0199 4.9410	+0.995 -1.180	100.995 98.820	1.012 1.100

Mean value of five determinations.

The results obtained by the proposed method were compared with British Pharmacopoeia (BP) method [Table (5)] by applying the F-test and the t-test at 95% confidence level. The calculated values for F (1.196) and t (0.793) for proposed method, did not exceed the critical values of $F_{7,7} = 4.995$ and $t = 2.14$ ($n_1 + n_2 - 2 = 14$). These confirming that there are no significant differences between the proposed method with BP method (23) with respect to precision and accuracy in the determination of paracetamol in pharmaceutical preparations.

Table (5)
Comparison of the proposed method with BP method for determination of paracetamol in pharmaceutical preparations..

Pharmaceutical preparation	Recovery, %*	
	Proposed method	BP method
Pure paracetamol	100.000	100.000
Paracetol tablets	99.215	98.881
Panadol extra tablets	99.410	100.726
Panatol tablets	99.270	99.025
Emidol tablets	99.125	99.242
Kanagesic tablets	101.110	98.700
Hayamol injections	99.520	99.314
Panatul suppositories	99.908	100.186

***Mean value of five determinations.**

Conclusion

Determination of paracetamol by proposed method is based on oxidation–reduction reaction by iron(III) salts. The proposed method is found to be simple, fairly, economical and highly sensitive than some of the reported methods. It has the advantage of being accurate, does not require the removal of excipients, temperature control, pH control, solvent extraction step and pre-hydrolysis step. The statistical parameters and recovery study data clearly indicate the reproducibility and accuracy of the method. It was applied successfully to pharmaceutical preparations.

References

[1] "The Merck Index on CD-ROM", Whitehouse Station, NJ, USA, 12th ed., 2000.

[2] J. S. E. Romero, L. A. Rodriguez, M. C. G. Alvarezcoque and G. Ramisramos, "Spectrophotometric determination of phenols by coupling with diazotized 2,4,6-trimethylaniline in amicellar medium", Analyst, Vol. 119, No. 6, 1994, pp. 1381 – 1386.

[3] N. Erk and F. Onur, "Simultaneous determination of analgine and paracetamol in tablets by spectrophotometric methods", Anal. Lett., Vol. 30, No. 6, 1997, pp. 1201 – 1210.

[4] F. A. Mohamed, M. A. Abdallah and S. M. Shammat, "Selective spectrophotometric determination of p-aminophenol and acetaminophen", Talanta, Vol. 44, No. 1, 1997, pp. 61 – 68.

[5] M. Nogowska, I. Muszalska and M. Zajac, "Simultaneous spectrophotometric determination of acetylsalicylic acid, paracetamol and caffeine in pharmaceutical preparations", Chem. Anal., Vol. 44, No. 6, 1999, pp. 1041 – 1048 .

[6] A. Afkhami and N. Sarlak, "Spectrophotometric determination of salicylamide and paracetamol in mixture by partial least squares regression (PLS) and H-point standard addition method (HPSAM)", Acta Chim. Slov., Vol. 25, 2005, pp. 98-103.

[7] A. Afkami, N. Sarlak and A. R. Zerei, "Spectrophotometric determination of salicyamide and paracetamol in biological samples and pharmaceutical formulations by a differential kinetic method", Acta Chim. Solv., Vol. 53, 2006, pp. 357 – 362 .

[8] M. M. Sena and R. J. Poppi, "N-way PLS applied to simultaneous spectrophotometric determination of acetylsalicylic acid, paracetamol and caffeine", J. Pharm. Biomed. Anal., Vol. 34, No. 1, 2004, pp. 27 – 34.

[9] M. I. Walash, A. M. Elbrashy and M. A. Sultan, "Polarographic behaviour and determination of paracetamol and salicylamide after treatment with nitrous acid", Microchim. Acta., Vol. 113 No. (1-2), 1994, pp. 113 – 124.

[10] S. Boonkerd, M. Lauwers, M. R. Detaevernier and Y. Michotte, "Separation and simultaneous determination of the components in an analgesic tablet formulation by micellar electrokinetic chromatography", J. Chromatogr., Vol. 695, No. 1, 1995, pp. 97 – 102.

[11] J. A. M. Pulgarin and L. F. G. Bermejo, "Flow-injection stopped-flow spectrofluorimetric kinetic determination of paracetamol based on its oxidation reaction by hexacyanoferrate(III)", Anal. Chim. Acta., Vol. 333, No. (1-2), 1996, pp. 59 – 69.

[12] Z.Bouhsain, S.Garrigues, A. Moralesrubio and M. Delaguardia, "Flow injection Fourier-transform infrared spectrometric determination of paracetamol in pharmaceuticals", Analyst, Vol. 121, No. 5, 1996, pp. 635 – 639.

[13] Z. Bouhsain, S. Garrigues and M. Delaguardia, "Flow injection spectro-photometric determination of paracetamol in pharmaceuticals by means of on-line microwave-assisted hydrolysisand reaction with 8-hydroxyquinoline (8-quinolinal)", Anal. Chim. Acta., Vol. 330, No. 1, 1996, pp. 59 – 69.

[14] A. Criado, S. Cardenas, M. Gallego and M. Valcarcel, "Continuous flow spectrophotometric determination of paracetamol in pharmaceuticals following continuous microwave assisted alkaline hydrolysis", Talanta, Vol. 53, No. 2, 2000, pp. 417 – 423.

[15] J. A. Marillo and L. F. Garcia, "Application of first derivative fluorescence spectrometry to the simulaneous determination of paracetamol and salicylamide in pharmaceuticals", Anal. Lett., Vol. 29, No. 3, 1996, pp. 423 – 438.

[16] J. M. Zen and Y. S. Ting, "Simultaneous determination of caffeine and acetaminophen in drug formulations by square-wave voltammetry using a chemically modified electrode", Anal. Chim. Acta., Vol. 342, No. (2 – 3), 1997, pp. 175 – 180.

[17] A. P. Argekar and J. G. Sawant, "Simultaneous determination of paracetamol and mefenamic acid in tablets by HPTLC", JPC – J. Planar Chromatogr.–Mod. TLC., Vol. 12, No. 5, 1999, pp. 361 – 364.

[18] V.Pucci, R.Mandrioli, M.A.Raggi and S. Fanali, "Reversed-phase capillary electrochromatography for the simultaneous determination of acetylsalicylic acid, paracetamol, and caffeine in analgesic tablets", Electrophoresis, Vol. 25, No. (4 – 5), 2004, pp. 615 – 621.

[19] M. Q. Al-Abachi and R. S. Al-Abaidi, "Spectrophotometric method for folic acid assay in pharmaceutical tablets", National J. of Chem., Vol. 8, 2002, pp. 527 – 534.

[20] A. Criado, S. Cardenas, M. Gallego and M. Valcarcel, "Fast urinary screening for paracetamol using on-line microwave assisted hydrolysis and spectrophotomrtric

detection", Analyst, Vol. 125, No. 6, 2000, pp. 1179 – 1183.
[21] A.I. Vogal, "A Text – Book of Macro and Semimicro Qualitative Inorganic Analysis", Longmans. London, 4th ed., 1954, pp. 261.
[22] J. N. Miller and J. C. Miller, "Statistics and Chemometrics for Analytical Chemistry", Pearson Education Limited, London, 4th ed., 2000.
[23] "British Pharmacopoeia on CD-ROM", The Stationery Office Ltd., London, 3rd ed., 2001.

الخلاصة

يتضمن البحث تطوير طريقة طيفية بسيطة وحساسة لتقدير الباراسيتامول باستخدام المطياف الضوئي. تعتمد الطريقة على تفاعل الباراسيتامول مع كبريتات الحديديك المائية ثم تفاعل ايونات الحديدوز الناتجة مع بوتاسيوم سيانيد الحديديك لتكوين ناتج أزرق اللون يمتلك أقصى امتصاص عند طول موجي 710 نانومتر. كان مدى الخطية 0.1 – 7.0 مايكروغرام مل$^{-1}$ باراسيتامول وحد الكشف 0.038مايكروغرام مل$^{-1}$ و قيمة الامتصاصية المولارية مساوية إلى 3.477×10^4 لتر مول$^{-1}$ سم$^{-1}$ وقيمة حساسية ساندل 4.348 نانوغرام سم$^{-2}$. بلغ الخطأ النسبي للطريقة اقل من ± 1.5% وانحراف قياسي نسبي 0.745 – 1.002% اعتماداً على تركيز الباراسيتامول. طبقت الطريقة المقترحة بنجاح لتقدير الباراسيتامول في المستحضرات الصيدلانية، وتم مقارنة النتائج مع الطريقة القياسية المعتمدة من قبل الدستور البريطاني لتقدير الادوية، ووجد ان الطريقتين القياسية والمقترحة لا تختلفان معنويا في الدقة والمصداقية.