METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF PARACETAMOL IN HUMAN PLASMA BY UV SPECTROPHOTOMETRY

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ABSTRACT: A rapid method for the determination of paracetamol in human blood serum using first derivative absorption spectroscopy is described. It involves no sample treatment, extraction or derivatization procedures, other than a standard deproteinizing technique with trichloroacetic acid. The percentage of relative recovery and coefficient of variation of accuracy and precision were within acceptable limits. The method proved in simple, costeffective, and sensitive foe estimation of Paracetamolin human plasma.This result can be applied to both therapeutic and toxic levels of Paracetamol.

KEYWORDS: Paracetamol, human plasma, method development, validation, bioanalytical procedure.

1.INTRODUCTION:

Paracetamol (N-acetyl-p-aminophenol) is one of the most popular analgesic,antipyretic drugs in current use. Although it has proven to be an extremely safe drug at therapeutic doses,large overdoses may be toxic [(1-3)]. In overdosepatient's plasma concentration may range from, 30to500µg mL^{-1} and they exhibit a drug half-life greater than the normal twohours. The most frequently reported adverse side effects associated with paracetamol is hepatotoxicity, which occurs after acute overdosage and very rarely during long term treatment with doses at higher levels of the therapeutic range.

Paracetamol is generally safe at recommended doses. The recommended maximum daily dose for an adult is 3 or 4 grams.Higher doses may lead to toxicity, including liver failure.Serious skin rashes may rarely occur. It appears to be safe during pregnancy and when breastfeeding. In those with liver disease, it may still be used, but in lower doses.It is classified as a mild analgesic. It does not have significant anti-inflammatory activity. How it works is not entirely clear [(4-5)]

Paracetamol was first made in 1877.It is the most commonly used medication for pain and fever in both the United States and Europe. It is on the World Health Organization's List of Essential Medicines, which lists the most effective and safe medicines needed in a health system.Paracetamol is available as a generic medication with trade names including Tylenol and Panadol, among others.The wholesale price in the developing world is less than US$ 0.01 per dose. In the United States, it costs about US$0.04 per dose.In 2016, it was the 17th most prescribed medication in the United States, with more than 29 million prescriptions.*4*-Aminophenol may be obtained by the amide hydrolysis of paracetamol. *4*-Aminophenol prepared this way, and related to the commercially available Metol, has been used as a developer in photography by hobbyists. This reaction is also used to determine paracetamol in urine samples: After hydrolysis with hydrochloric acid, *4*-aminophenol reacts in ammonia solution with a phenol derivate, e.g. salicylic acid, to form an indophenol dye under oxidization by air[(11-20)].

IUPAC NAME OF PARACETAMOL:

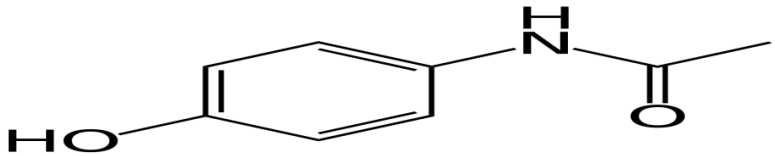

FIGURE1: STRUCTURE OF PARACETAMOL

Paracetamol consists of a benzene ring core, substituted by one hydroxyl group and the nitrogen atom of an amide group in the para pattern. The amide group is acetamide (ethionamide) [(4-5)]

CATEGORY: ANALGESIC, ANTIPYRETIC
MOLECULAR FORMULA: C8H9NO2
MOLECULAR WEIGHT:155.189 g/mol
MOLAR MASS: 151.163 G/MOL
MELTING POINT: 169 °C
BOILING POINT: 420 °C (788 °F)
DENSITY: 1.26 G/CM³
METABOLISM: Predominantly in the liver [(4-10)]

MECHANISM OF ACTION OF PARACETAMOL:

Paracetamol (acetaminophen) is generally considered to be a weak inhibitor of the synthesis of prostaglandins (PGs). However, the in vivo effects of paracetamol are similar to those of the selective cyclooxygenase-2 (COX-2) inhibitors. Paracetamol also decreases PG concentrations in vivo, but, unlike the selective COX-2 inhibitors, paracetamol does not suppress the inflammation of rheumatoid arthritis. It does, however, decrease swelling after oral surgery in humans and suppresses inflammation in rats and mice. Paracetamol is a weak inhibitor of PG synthesis of COX-1 and COX-2 in broken cell systems, but, by contrast, therapeutic concentrations of paracetamol inhibit PG synthesis in intact cells in vitro when the levels of the substrate arachidonic acid are low. When the levels of arachidonic acid are low, PGs are synthesized largely by COX-2 in cells that contain both COX-1 and COX-2. Thus, the apparent selectivity of paracetamol may be due to inhibition of COX-2-dependent pathways that are proceeding at low rates. This hypothesis is consistent with the similar pharmacological effects of paracetamol and the selective COX-2 inhibitors. COX-3, a splice variant of COX-1, has been suggested to be the site of action of paracetamol, but genomic and kinetic analysis indicates that this selective interaction is unlikely to be clinically relevant. There is considerable evidence that the analgesic effect of paracetamol is central and is due to activation of descending serotonergic pathways, but its primary site of action may still be inhibition of PG synthesis. The action of paracetamol at a molecular level is unclear but could be related to the production of reactive metabolites by the peroxidase function of COX-2, which could deplete glutathione, a cofactor of enzymes such as PGE synthase [1-3]

2. INTRODUCTION TO UV SPECTROSCOPY

UV spectroscopy is type of absorption spectroscopy in which light of ultra-violet region (200-400 nm.) is absorbed by the molecule. Any molecule has either n, π or σ or combination of these electrons. These bonding (σ and π) and non-bonding(n) electrons absorb the characteristic radiation and undergoes transition from ground state to excited state. By the characteristic absorption peaks and the nature of the electron present the molecular structure can be elucidated

UV spectroscopy obeys the Beer-Lambert law,

Beer law: This law can be stated as follows: "When a beam of monochromatic radiation is passed through a solution of absorbing substances, the intensity of a beam of monochromatic light decreases exponentially with the increase in concentration of the absorbing substances exponentially".

$I=I_0*e^{-k1*c}$ ——— 1

Where, I_0 =intensity of light incident upon sample cell
I=intensity of light leaving sample cell
C= molar concentration of solute
K_1=constant

Lambert's law: This law can be stated as follows "When a beam of light is allowed to pass through a transparent medium, the rate of decrease of intensity with the thickness of medium is directly proportional to the intensity of the light".

$I=I_0*e^{-k2*l}$ ——— 2

Where, I_0= intensity of light incident upon sample cell
I = intensity of light leaving sample cell
L = length of sample cell (cm.)
K_2=constant

After combining equation 1 and 2 and deriving we get the following equation 3 of Beer-Lambert law as:

$A = \log (I_0/I) = \varepsilon cl$ ——— 3

Where, A = absorbance
I_0 = intensity of light incident upon sample cell
I = intensity of light leaving sample cell
C = molar concentration of solute
L = length of sample cell (cm.)
Ɛ = molar absorptivity

A literature search has shown that there are only few quantitative analyticalmethods for estimation of paracetamol further, very few methods were available that shows the quantification of paracetamolin biologicalfluids, these methods include LC-MS, GC-MS, which needs high end instrumentation which are costly and not available in conventionalbioanalytical laboratory. Thus, the conclusion was to develop a rapid, simpleandeconomical method which wasbased on liquid–liquid extraction (LLE) for sample preparationand UV detection for quantification of paracetamol from spiked human plasma.

3.MATERIALS AND METHODS:

CHEMICALS AND REAGENTS: PARACETAMOL(N-ACETYL-P-AMINOPHENOL)

4.Instrumentation

Double beam UV spectrophotometer; Model: SL 210; Make: ELICO.The data was obtained using Spectra Treats 3.11.01Rel 2b
Vortex mixer; Model: CM101; Make: REMI

THE ANALYSIS WAS PERFORMED USING UV SL120 USING UV DETECTOR USED FOR METHOD DEVELOPMENT AND VALIDATION. THE OUTPUT SIGNAL WAS CHECKED AND THE ACQUISITION AND INTEGRATION OF DATA WAS PERFORMED USING SPECTRAL THREATS. SOFTWARE ON A COMPUTER. THE DILUENTS ARE FILTERED THROUGH 0. 25MM.DETECTION was monitored at 292nm.

5.Procedure

5.1Selection of wavelength

10mg of paracetamol drug was accurately weighed and transferred into 10 ml of volumetric flask and the volume was made up to the mark with distilled water as diluent .Then from this 0.1 ml was pipetted out and transferred into another 10 ml volumetric flask and the volume was made up to the mark with distilled to give 10ppm solution and this was scanned between 200 to 400nm and its absorbance was measured at 257nm.

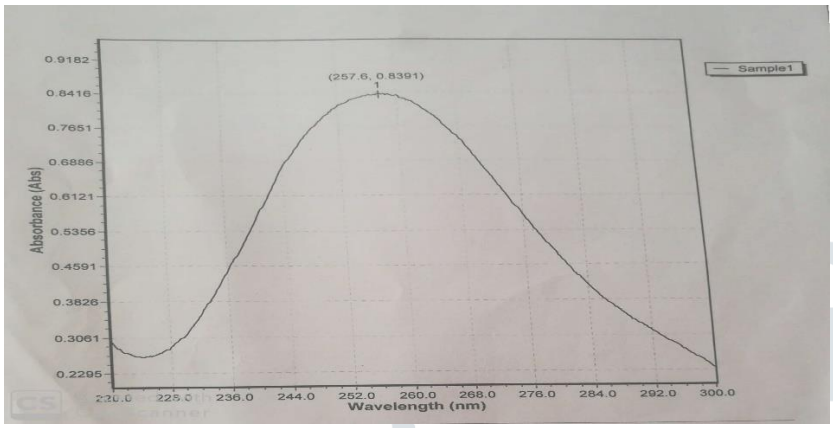

Figure 2: UV spectrum of Paracetamol

5.2Assay

5.2.1Standard preparation

10mg of paracetamol drug was accurately weighed and transferred into 10ml of volumetric flask and the volume was made up to the mark with distilled water to get concentration of 1000ppm.From this 0.1 ml was pipetted out and transferred into 10ml of volumetric flask and the volume was made up to the mark with distilled to get 10ppm solution and its absorbance was measured at 225nm.

5.2.2Test preparation

20 tablets were weighed and powdered. Powdered tablet equivalent to 10 mg of paracetamol was weighed accurately and it was taken into 10ml volumetric flask then volume was made up to the mark with methanol. Fromthe above solution 0.1 ml of solution was pipetted out and taken in 10ml volumetric flask. The volume was made up to 10ml to get 10ppm solution and its absorbance was measured at 225nm.

The % Assay is calculated by using the following formula

% Assay= ((absorbance of the sample/absorbance of the standard) *(concentration of the standard/concentration of the sample)) *100

5.2.3Preparation of standard stock solutions:

Preparation of standard solution: accurately weighed 10mg of paracetamol was transferred into 10ml volumetric flask, dissolved and made up to the mark with diluent. This was the solution having strength of 1000µg/ml of paracetamol.

6.Extraction of plasma from blood:

Blood was collected into an EDTA containing tube and then it was centrifuged for10min at 3000rpm.blood was separated into layers after centrifugation. The supernatant which contains stray yellow color (plasma) was collected and used for sample preparation.

6.1Preparation of plasma solution:

To the samples of serum appropriate aliquots of th paracetamol stock solution were added. Deproteinization was achieved by adding 2ml of trichloroacetic acid. After centrifuging,1ml of supernatant solution was treated with 0.2ml of 10M NaoH. The UV absorption spectrum was recorded and the absorbance was checked at 292 nm

7.Method validation parameters

Method validation: ICH guidance for industry was followed for validation of the method. Linearity, Accuracy, Robustness, LOD, LOQ were assessed during method validation.

7.1. Linearity

Calibration standard solutions were prepared in plasma from the working solutions. Five calibration curves ranging from the 2 to 10 ppm were run to establish the linearity by using linear regression analysis. From the stock solution 0.2ml, 0.4 ml, 0.6 ml, 0.8 ml and 1.0 ml was pipetted out and transferredinto 10ml volumetric flask and the volumewas made up to 10ml with methanol to give 2ppm, 4ppm, 6ppm. 8ppmand 10ppm concentration. Respectively and absorbance was measured at 292nm using distilled water as blank and the calibration curve is plotted.

7.2. Precision

10ppm standard solution ofparacetamol pure drug is selected for Precision study. From the standard stock solution 0.1ml was pipetted out and transferred into 10ml volumetric flask and the volume was made up to 10ml using distilled water to give 10ppm solution. This procedure is repeated 6 time and observances of all were measured at 223nm using distilled water as blank and its %RSD was calculated by using the formula

%RSD = (standard deviation of the measurement / mean value of measurement) *100

7.3. Accuracy

Quality control of samples was prepared at four different levels. The concentration of paracetamol was calculated from a standard calibration curve that was concurrently obtained. Accuracy was analyzed at each level by comparing the observed concentration as a mean relative percentage recovery.Standard quantity equal into 50%, 100% and 150 % is to be added in sample.2ml of standard solution was spiked with 4ml of sample solution,2ml of standard solution was spiked with 6ml of sample solution,2ml of standard solution was spiked with 8ml of sample solution. Absorbance was measured for three times at 292nm.

repeated three times and their absorbance is measured at 292nm and the %recovery is calculated by using the formula:

% recovery = (amount found / amount added) *100

7.4.Limit of detection

The detection limit (DL) may be expressed as:

$$DL = 3.3*\sigma/ S$$

where σ = the standard deviation of the response S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

7.5.Limit of quantification

The quantitation limit (QL) may be expressed as:

$$QL = 10*\sigma/ S$$

where σ = the standard deviation of the response S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

7.6.Robustness

Robustness:6 aliquots of 6ppm of standard solution was prepared and it was scanned at wavelength at (±)1nm of λmax. The absorbance was noted down.

8.Results and discussion:

Method development and optimization of chromatographic condition:

The %assay was found to be 99%.

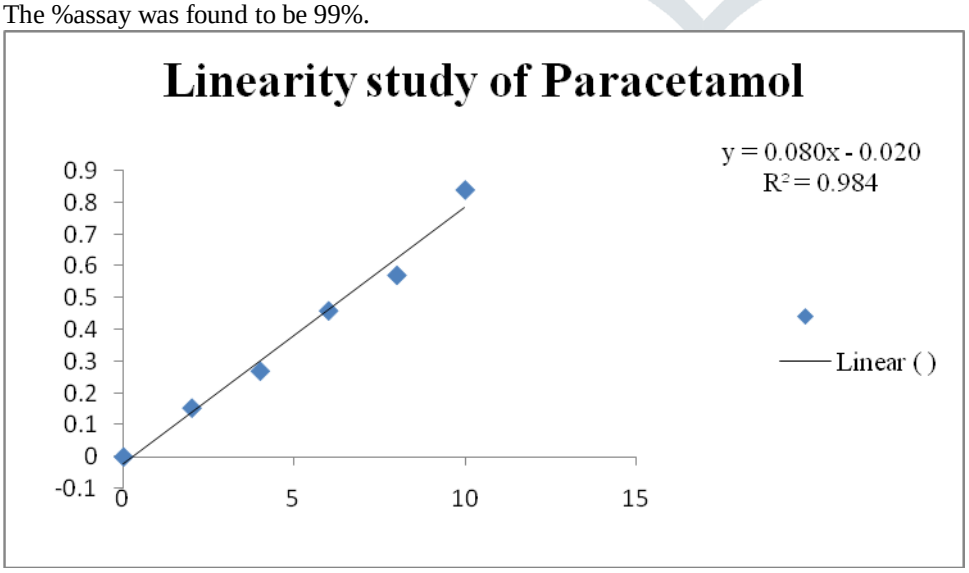

Figure:2linearity curve of paracetamol

Concentration(ppm)	Absorbance(nm)
0	0
2	0.1536
4	0.2797
6	0.4591
8	0.5708
10	0.8391

Table-1: Conc. Vs Abs. table for Linearity Study

Sample no.	%RSD
1	0.3597
2	0.3595
3	0.3589
4	0.3672
5	0.3572
6	0.3664
Mean	0.3582
SD	0.001390
%RSD	0.3881

Table-2: Evaluation data of precision study

% Recovery level	%Recovery	Mean % recovery
50%	99.55	99.56
	99.65	
	99.50	
100%	99.68	99.71
	99.79	
	99.68	
150%	99.86	99.86
	99.75	
	99.90	

Table-3: Evaluation data of accuracy study.

The limit of detection was found to be 0.51 ppm and limit of quantification found to be 1.57ppm.

Sample no.	222nm	223nm	224nm
1	0.7289	0.7462	0.7490
2	0.7289	0.7461	0.7492
3	0.7285	0.7458	0.7487
4	0.7284	0.7458	0.7484
5	0.7285	0.7453	0.7486
6	0.7281	0.7456	0.7485
Mean	0.7286	0.7458	0.7487
SD	0.0003082	0.0003286	0.0003077
%RSD	0.04230	0.04406	0.04110

Table-4: Evaluation data of robustness study.

9.Acknowledgment

We are thankful that RBVRR Women's College of Pharmacy has provided us the opportunity to write this research article.

References

[1].Graham GG[1], Scott KF;et.al Am J Ther. ACETAMINOPHEN - Hazardous Substances Data BankDrug Bank: Data: Chemical - SciCrunch 2005 Jan-Feb; vol 12(1):pg.no:46-55.

[2]. Scott KF, Bryant KJ, Bidgood MJ. J Leukoc Biol; Et.al. Functional coupling and differential regulation of the phospholipase A2-cyclooxygenase pathways in inflammation.1999 Oct; vol.66(4): pg.no535-41.

[3]. Graham GG, Scott KF;et.al Mechanisms of action of paracetamol and related analgesics. Inflammopharmacology. 2003; vol.11(4): pg.no401-13.

[4].McEvoy,G.K. (ed);et.al American Hospital Formulary Service Drug Information 95. Bethesda, MD: American Society of Hos pitalPharmacists, Inc., 1995 (Plus Supplements 1995)., pg.no. 1417

[5].Budavari S;et.al, The Merck Index Encyclopedia of Chemicals, Drugs and Biologicals. Rahway, NJ: Merck and Co., Inc., 198 9., pg.no.1323

[6]. MARTA J" èWIAK-B BENISTA* and JERZY Z. NOWAK; et.al. Acta Polonia Pharmaceutical Drug Research, Vol.no:71 pg.no. 11-23, 2014

[7]. Ghanem CI, Gómez PC, Arana MC, Perassolo M, Ruiz ML, Villanueva SS, Ochoa EJ, Catania VA, Bengochea LA, Mottino AD; et.al. Biochem Pharmacol. 2004 Aug 15;68(4):791-8

[8]. Ghanem C, Gomez PC, Arana C, Bengochea L; et.al. Acta Gastroenterol Latinoam. 2002; vol.32(2):pg.no79-82. Spanish.

[9]. Nick Buckley et.al., Consultant Clinical Pharmacologist and Toxicologist# and Michael EddlestonBMJ Clin Evid. 2007; 2007: 2101

[10]. Vale JA, lancet et.al; Proudfoot AT. Paracetamol (acetaminophen) poisoning. 1995; pg.no346:547–552.

[11]. Clark R, Borirakchanyavat V, Davidson AR, et al. Hepatic damage and death from overdose of paracetamol. *Lancet* 1973; 1:66–70.

[12]. Prescott LF. Paracetamol overdosage. Pharmacological considerations and clinical management. *Drugs*1983; vol.25: pg.no290–314

[13]. Mahadevan SB, McKiernan PJ, Davies P, et al. Paracetamol induced hepatotoxicity. *Arch Dis Child*2006; 91: pg.no 598–603.

[14]. *De Martino, Maurizio; Chiarugi, Alberto et.al. (2015). "Recent Advances in Paediatric Use of Oral Paracetamol in Fever and Pain Management". Pain and Therapy. Vol.**4** (2): 149–168.*

[15].Bailey, E; Worthington, H; Coulthard, P et.al."Ibuprofen and/or paracetamol (acetaminophen) for pain relief after surgical removal of lower wisdom teeth, a Cochrane systematic review". *British Dental Journal*. **216** (8): pg.no: 451–5(April 2014).

[16]. *Larson AM; Polson J; Fontana RJ; et al. (2005). "Acetaminophen-induced acute liver failure: results of a United States multicentre, prospective study". Hepatology. Vol.**42** (6): pg.no1364–72.*

*[17]. Villar D, Buck WB, Gonzalez JM; et.al (1998). "Ibuprofen, aspirin and acetaminophen toxicosis and treatment in dogs and cats". Vet Hum Toxicol. Vol.**40** (3):pg.no156–62.*

[18].Toussaint, K.; Yang, X.C.; Zielinski, M.A.; Riegle, K.L.; Scavenge, S.D.; Nagar, S.; Raffa, R.B; et.al (December 2010). "What do we (not) know about how paracetamol (acetaminophen) works?". *Journal of Clinical Pharmacy and Therapeutics*. Vol.**35** (6): pg.no617–638.

[19]. Rumack Matthew H (1975)."Acetaminophen poisoning and toxicity". Paediatrics.Vol.**55** (6):pg.no871–76.

[20]. Moore, RA; Derry, C (January 2013). "Efficacy of OTC analgesics". *International Journal of Clinical Practice. Supplement*. Vol.**67** (178): pg.no 21–5.