WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 9, 1659-1685. Research Article ISSN 2277– 7105

IMPURITY PROFILING OF PARACETAMOL DOSAGE FORMS USED IN MAIDUGURI METROPOLIS

Hassan Yesufu Braimah*[1], Abubakar Babakura Tijjani[1] and Samuel Chabiri Amos[2]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Maiduguri, Maiduguri, Nigeria.

[2]National Agency for Food Drug Administration and Control Maiduguri Area Laboratory, Maiduguri, Nigeria.

Article Received on
21 June 2019,
Revised on 11 July 2019,
Accepted on 01 Aug. 2019
DOI: 10.20959/wjpr20199-15598

***Corresponding Author**
Hassan Yesufu Braimah
Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Maiduguri, Maiduguri, Nigeria.

ABSTRACT

Pharmaceutical impurities are those substances which co-exist with the active pharmaceutical ingredient (API) or they may develop during synthesis or ageing of both API and formulation. The presence of these impurities even in minor amounts can influence the efficacy and safety of drug. Paracetamol (N-(4-hydroxyphenyl) acetamide) also known as acetaminophen is a widely used analgesic for management of pain and fever in a variety of patients including children, pregnant women and the elderly. The aim of this work was to test for the presence of impurities in paracetamol dosage forms used in the Maiduguri metropolis by TLC and HPLC methods. For PAP detection an Intersil ODS-3V column (150 mm × 4.6 mm; 5 µm pore size) was used at the temperature of 35^0C, the mobile phase was methanol : water (20 : 80) using isocratic elution at the flow rate of 2 ml/min, injection volume of 10 µl and a diode array detector. For PCA detection, the chromatographic conditions were the same except the mobile phase which was methanol: water (60: 40), flow rate of 1.2 ml/min and injection volume of 20 µl. 15 samples each of paracetamol tablet and syrups were randomly selected from three different sources; hospital pharmacy, community pharmacy and drug patent stores. Using the TLC method none of the samples showed spots corresponding to PAP or PCA. Using the HPLC method, one sample of paracetamol syrup presented with a peak that corresponds to PAP and upon quantification the amount was below the limit stated by the pharmacopoeia thus the sample was accepted. None of the samples showed peak corresponding to PCA. corresponding to PCA.

KEYWORDS: Pharmaceutical impurity, paracetamol, HPLC, TLC, PAP and PCA.

Abbreviations: USP: United States Pharmacopoeia, BP: British Pharmacopoeia, HPLC: High Performance Liquid Chromatography, TLC: Thin Layer Chromatography; PTH: Paracetamol Tablet Hospital sample, PSH: Paracetamol Syrup Hospital sample; PTCP: Paracetamol Tablet Community Pharmacy sample, PSCP: Paracetamol Syrup Community Pharmacy sample, PTPS:Paracetamol Tablet Patent Store sample and PSPS: Paracetamol Syrup Patent Store sample.

INTRODUCTION

Pharmaceutical impurities are those substances which co-exist with the active pharmaceutical ingredient (API) or they may develop during synthesis or ageing of both API and formulation. The presence of these impurities even in minor amounts can influence the efficacy and safety of drug. The safety of drug is dependent not only on the toxicological properties of active drug substance itself, but also on impurities that it contains. As safety and quality of pharmaceutical products can be affected by impurities present in APIs, impurity profiling of API has started gaining wider attention.[1] Impurity profiling (i.e. the identity as well as the quantity of impurity in the pharmaceuticals), is now receiving important critical attention from regulatory authorities. The different pharmacopoeias, such as the British Pharmacopoeia (BP) and the United States Pharmacopoeia (USP), are slowly incorporating limits to allowable levels of impurities present in the APIs or formulations.[2] Development of analytical methods to determine impurities and degradation products during pharmaceutical development is necessary.[3]

It is virtually impossible to have absolutely pure chemical compounds and even analytical pure chemical compounds contain minute trace of impurities. The chemical purity may be achieved as closely as desired provided that sufficient care is observed at different levels in manufacturing of a pharmaceutical.[4] The level of purity of the pharmaceutical substances depends partly on the cost-effectiveness of the process employed, method of purification and stability of the final product. Setting higher standards of purity for pharmaceutical substances than that of desirable and pharmacologically safe level will unduly result in wastage of money, material and time. Purification of chemical compounds is a very expensive process hence one has to strike a balance in order to obtain a pharmaceutical substance at reasonable cost yet sufficiently pure for all pharmaceutical purposes.[5]

Paracetamol

Paracetamol (N-(4-hydroxyphenyl)acetamide) also known as acetaminophen is a widely used analgesic for management of pain and fever in a variety of patients including children, pregnant women and the elderly.[6] Paracetamol, is often the analgesic or antipyretic of choice in patients in whom salicylates or other NSAIDs are contra-indicated; asthmatics or those with a history of peptic ulcer, or children in whom salicylates are contra-indicated because of the risk of Reye's syndrome.[7] Paracetamol is hydrolyzed to p-aminophenol and acetic acid. Some impurities originate from the synthesis pathway like p-chloroacetanilide.[8]

Different assay methods are available for the evaluation of paracetamol in bulk and in dosage forms. USP has described an analytical method to quantify paracetamol as a bulk drug by spectrophotometry at 244 nm using water as blank. Paracetamol tablets are assayed by liquid chromatography using a mixture of water and methanol (3 :1) as mobile phase and solvent system.[9] The British Pharmacopoeia also have spelt out methods.[10] Different paracetamol analysis methods published in over 50 years were summarized in a review article.[11]

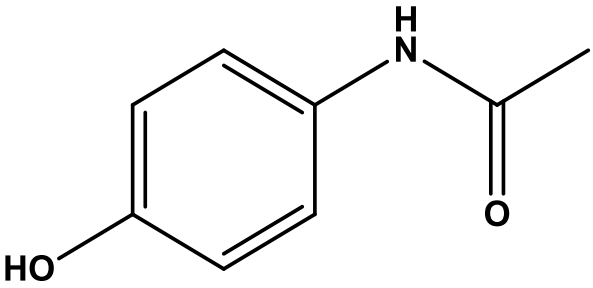

Fig. 1: Paracetamol.

Impurities in Paracetamol

P-aminophenol

P-aminophenol (PAP) is an analog and metabolite of common household analgesics, such as acetaminophen. It is well-known that acetaminophen in overdose can cause severe hepatic centrilobular necrosis in humans and experimental animals.[12] P-aminophenol is a nephrotoxic metabolite of acetaminophen. It is 5 times more potent than acetaminophen as nephrotoxicant in F344 rats. Inhibition of acetaminophen de-acetylation to p-aminophenol diminished renal toxicity, suggesting that acetaminophen renal toxicity is partly mediated by formation of p-aminophenol. P-aminophenol nephrotoxicity is site-specific for the S3 segment of the proximal tubule.[13]

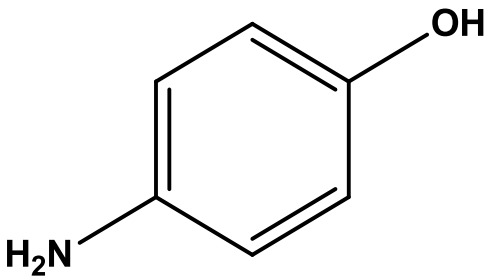

Fig. 2: P-aminophenol, a degradation product of paracetamol.

Assay of p-aminophenol

Limits of p-Aminophenol in USP are (NMT 0.005%) in bulk drug. The limit varies among different dosage forms, for tablets (NMT 0.005%), for suppositories (NMT 0.0005%) and for syrup (NMT 0.5%). P-aminophenol is analyzed in the USP by color development with alkaline nitroferricyanide solution, followed by absorption measurement at 710 nm. This reaction depends on replacement of nitrosyl ion with PAP in the complex.[9] Limits of P-Aminophenol in BP are (NMT 0.005%) in bulk drug, (NMT 0.1%) in paracetamol tablet and suppository and (NMT 0.5%) in paracetamol syrup. According to BP, p-aminophenol is detected by liquid chromatography using a mixture of 250 volumes of methanol containing 1.15 g of a 40% (v/v) solution of tetrabutylammonium hydroxide with 375 volumes of 0.05M disodium hydrogen orthophosphate and 375 volumes of 0.05M sodium dihydrogen orthophosphate as mobile phase and a detection wavelength of 245nm.[10]

A rapid and simple spectrophotometric method was applied for quantification of urine PAP concentration using a chemical auto analyzer. The method involved oxidative coupling of PAP with an aromatic compound (Berthelot reagent). Xylenol and sodium periodate were applied for this purpose.[14]

A highly sensitive, precise and automated assay employing the technique of flow injection analysis to quantitatively assay low levels of PAP was developed and applied for paracetamol drug and tablet formulation. A solution of the drug substance or extract of the tablets is injected into a solvent carrier stream and merged on–line with alkaline sodium nitroprusside reagent to form a specific blue derivative which is detected spectrophotometrically at 710nm.[15]

P-chloroacetanilide

P-chloroacetanilide (PCA) is also an impurity of paracetamol that is often encountered in its impurity profiling studies. Chloroacetanilide herbicides are among the most commonly used herbicides in agriculture. Several studies have demonstrated a number of them to be carcinogenic. Inhalation and ingestion are the main routes of entry to the human body. In case of inhalation it causes lung irritation also causes irritation to the skin or the eyes by direct contact, it may affect genetic material and may cause digestive tract irritation. The properties of this substance have not been fully investigated.[16]

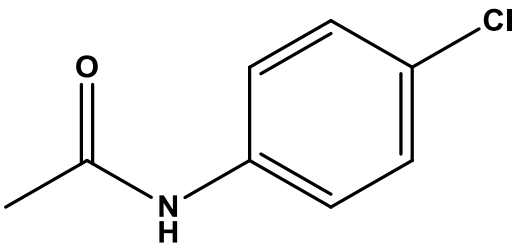

Fig. 3: P-chloroacetanilide, paracetamol related substance.

Assay of p-chloroacetanilide

Limit of p-chloroacetanilide is included in the USP (NMT 0.001%) and is analyzed by thin-layer chromatography using silica gel as stationary phase, while the mobile phase is hexane-acetone (75: 25).[9]

There are some other impurities found to be occurring in paracetamol samples but are less common, they mentioned in the BP;

- ✓ Paracetamol impurity A (N-(2-hydroxyphenyl) acetamide).
- ✓ Paracetamol impurity B (N-(4-hydroxyphenyl) propanamide.
- ✓ Paracetamol impurity C (N-(3-chloro-4-hydroxyphenyl) acetamide).
- ✓ Paracetamol impurity D (N-phenylacetamide).
- ✓ Paracetamol impurity E (1-(4-hydroxyphenyl) ethanone).
- ✓ Paracetamol impurity F (4-nitrophenol).
- ✓ Paracetamol impurity G (1-(4-hydroxyphenyl) ethanone oxime).
- ✓ Paracetamol impurity H (4- (acetylamino)phenyl acetate).
- ✓ Paracetamol impurity I (1-(2-hydroxyphenyl) ethanone).[10]

MATERIALS AND METHOD

Instruments

Hitachi HPLC machine; pump model L-2130 (USA), Intersil ODS-3V C-18 column; 150mm × 4.6mm; 5µm pore size (GL Sciences, USA), shaker and centrifuge.

Reagents

Standards: paracetamol and 4-chloroacetanilide, (Sigma Aldrich, France), 4-aminophenol (Merck, Germany). Solvents: methanol,(Sigma Aldrich, France), acetone, ethanol, toluene, acetic acid, ethyl acetate, hexane, and distilled water. Substances: iodine, and ammonia (BDH chemicals, UK). Materials: TLC plates Kiesel gel 60 F254 (5 ×10cm) (Merck, Germany), micro capillary 2µl.

Sampling

The sample were selected from the different pharmacy premises as well as the patent stores. Different dosage forms were used; tablets and syrups. The pharmacies and the patent stores of each area were selected randomly.

Sample Size

The samples were randomly selected from three different sources; hospitals, community pharmacies and patent drug stores. 300 tablets and 15 syrups of paracetamol produced by 5 different companies were randomly selected. Different doses and types were randomly selected.

Detection of Impurities of Paracetamol using TLC

Standard Solutions: For tablets

-Paracetamol standard solution: 1g standard paracetamol was dissolved in 5 ml methanol.

-Para-aminophenol standard solution: The limit of para-aminophenol is not more than 0.005% (USP, 2017 and BP, 2018). 0.05g of standard para-aminophenol was dissolved in 10ml methanol. 1ml of the resulting solution was diluted to 100ml with methanol to get a final concentration of 50µg/ml.[11]

-Para- chloroacetanilide standard solution: The limit of para-chloroacetanilide as an impurity in paracetamol is not more than 0.001%.[9,10] 0.01g of the standard para- chloroacetanilidewas dissolved in 10ml methanol. 1ml of the resulting solution was diluted to 100 ml with methanol. The resulting solution has a concentration of 10µg/ml.[11]

Standard Solution: For Syrups

-Paracetamol standard solution: 25mg paracetamol was dissolved in 5ml methanol. The concentration of the standard solution was 5mg/ml.[11]

-Para-aminophenol standard solution: The limit of para-aminophenol in paracetamol syrup is not more than 0.5% (USP, 2017 and BP, 2018). 25 mg of standard para-aminophenol was dissolved in 10ml methanol. 1ml of the resulting solution was diluted to 100ml with methanol to get a final concentration of 25µg/ml.[11]

-Para-chloroacetanilide standard solution: Para-chloroacetanilide standard solution with a concentration of 5.0µg/ml was prepared by dissolving 50mg of para-chloroacetanilide in 100ml methanol. 1ml of the last solution was transferred into 100ml volumetric flask and diluted to volume with methanol.[11]

A mixture of paracetamol, para-aminophenol and para- chloroacetanilide was prepared by mixing equal volumes of their standard solutions.

Chromatography

Stationary phase: TLC plates (Kiesel gel 60 F254, 5 × 10 cm).

Mobile phases: Different mobile phases were tested to get the best isolation for paracetamol samples along with its impurities by TLC. These will include:

Chloroform: acetone (4:1); ethyl acetate: hexane (3:1); Ethyl acetate : acetic acid (200:1); toluene : acetone : acetic acid (20:20:2); hexane : ethyl acetate (3:4) and toluene : acetonitrile (1:1).

Application: 10µl of each paracetamol standard solutions, 2µl of para-aminophenol, para chloroacetanilide, standard solutions and mixture solutions were applied. The spots were detected using iodine vapor. After identifying the best solvent system, the drug samples were prepared by accurately weighing powder equivalent to 1g paracetamol and dissolving in 5 ml methanol. It was mechanically shaken for 30 minutes and centrifuged at 1000 rpm for 15 minutes. The clear supernatant was then separated. This clear solution was usedfor spotting together with the solutions of the standards (paracetamol, para-aminophenol and para-chloroacetanilide). Commercial paracetamol syrup that has a concentration of 125 mg/5ml was examined. 5 ml of the preparation was diluted to 25 ml with methanol and filtered. The filtrate was used for spotting together with the standard solutions.

Evaluation: $R_f$values were determined. The spots of para-aminophenol and para-chloroacetanilide in paracetamol tablet samples should not be larger than the corresponding

para-aminophenol and para-chloroacetanilide spots in standard solution. (the limit of para-aminophenol is 0.005% and para chloroacetanilide 0.001%).The spots of para-aminophenol and para- chloroacetanilide in paracetamol syrup samples should not be larger than the corresponding para-aminophenol and para-chloroacetanilide spots in standard solution. (the limit of para-aminophenol is 0.5% and para chloroacetanilide 0.1%).

Detection of Impurities of Paracetamol using HPLC

Using the standard powders, solutions of paracetamol, 4-aminophenol and 4-chloroacetanilide were prepared. Various concentrations were used to obtain the calibration curve. 25 mg of each standard was dissolved in 2 ml of methanol and made up to 10 ml using distilled water. 1.5 ml of the resulting solution was diluted to 25 ml with distilled water. From the second solution 1, 0.8, 0.6, 0.4 and 0.2 ml were taken and diluted to 25 ml with distilled water to give 6 µg/ml, 4.8 µg/ml, 3.6 µg/ml, 2.4 µg/ml and 1.2 µg/ml respectively. Peak area was plotted against concentration to obtain the calibration curve. To quantify paracetamol and its impurities, samples to be analyzed were prepared to obtain 150 µg/ml concentration. For tablets, quantity of powdered sample containing 25 mg equivalent of paracetamol was weighed and dissolved in 2 ml methanol and made up to 10 ml with distilled water. After filtration of the resulting solution, 1.5 ml was taken and diluted to 25 ml with distilled water to yield 150 µg/ml concentration. For syrups, 1 ml was be measured and dissolved in 2 ml methanol and made up to 10 ml with distilled water. After filtration of the resulting solution, 1.5 ml was taken and diluted to 25 ml with distilled water to yield 150 µg/ml concentration.

The chromatographic conditions are:

Column: Intersil ODS-3V C-18

Column dimension: 150 mm × 4.6 mm; 5 µm pore size

Mobile phase: methanol : water (20 : 80)

Flow rate: 2 ml/min

Injection volume: 10 µl

Mode of elution: isocratic

Column temperature: 35 ^{0}C

Detector: diode array detector (DAD L-2455).[17]

For 4-chloroacetanilide, the chromatographic conditions are same but with the following differences:

Mobile phase: methanol : water (60 : 40)

Flow rate: 1.2 ml/min

Injection volume: 20 µl.[18]

RESULTS AND DISCUSSION

Detection of Impurities in Paracetamol Dosage Forms using TLC

All the examined paracetamol samples (tablets and syrups) have shown no spots corresponding to para-aminophenol (PAP) or para-chloroacetanilide (PCA) on the developed silica gel plates. The pictures below show representative chromatograms using hexane : ethyl acetate (3:4) as the mobile phase.

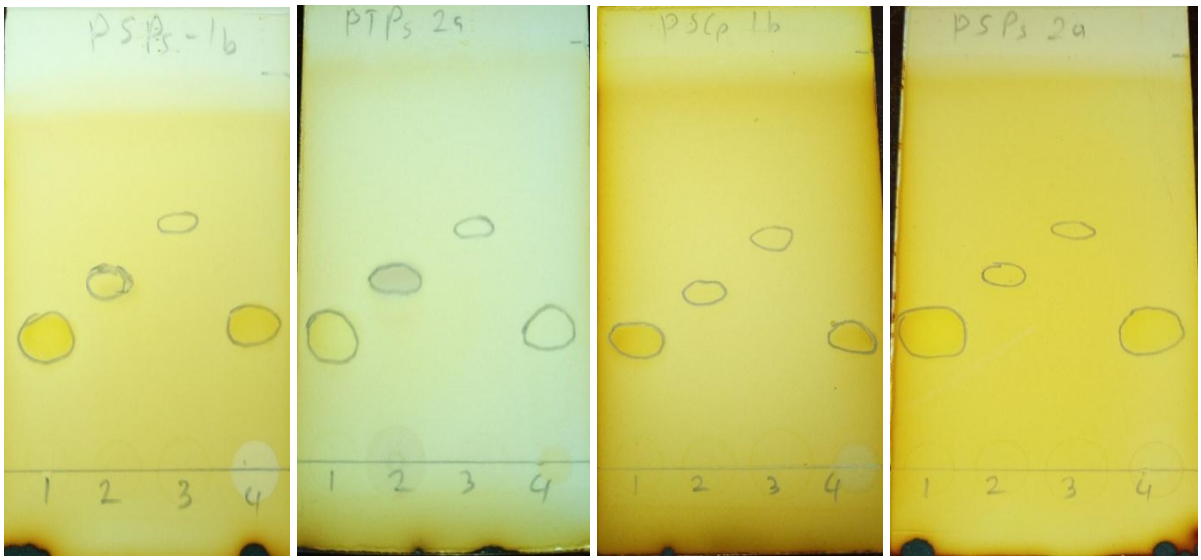

Fig. 4: TL Chromatograms of Sample. Key: 1-PCM STD, 2-PAP STD, 3-PCA STD and 4-Sample.

Detection of Impurities in Paracetamol Dosage Forms using HPLC

With regards to para-aminophenol all the examined paracetamol dosage forms have shown no peaks corresponding to para-aminophenol on the chromatograms except sample PSPS-3. However, after plotting the calibration curve for para-aminophenol and using it to quantify the impurity, it was found to be 0.00006% which is far below the USP limit (USP, 2017); thus it has passed the limit test for para-aminophenol. The chromatograms are shown below:

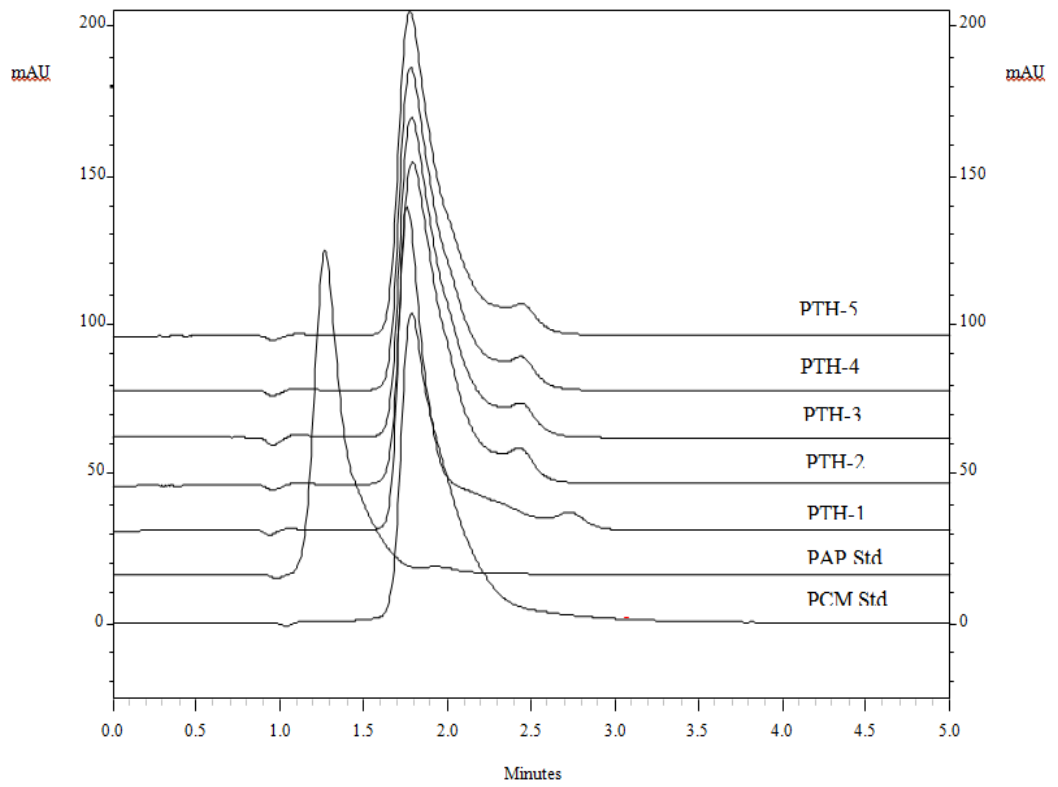

Fig. 5: HPL Chromatogram of PCM tabs Hospital Samples for PAP Profiling.

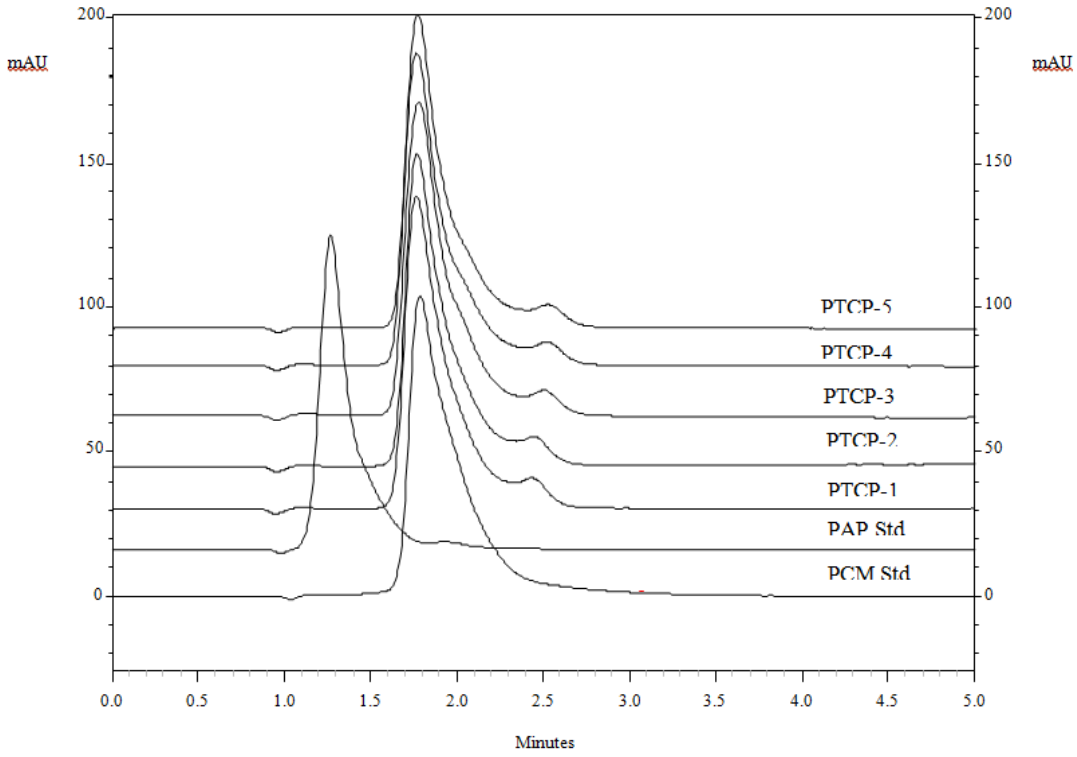

Fig. 6: HPL Chromatogram of PCM tabs Community Pharmacy Samples for PAP Profiling.

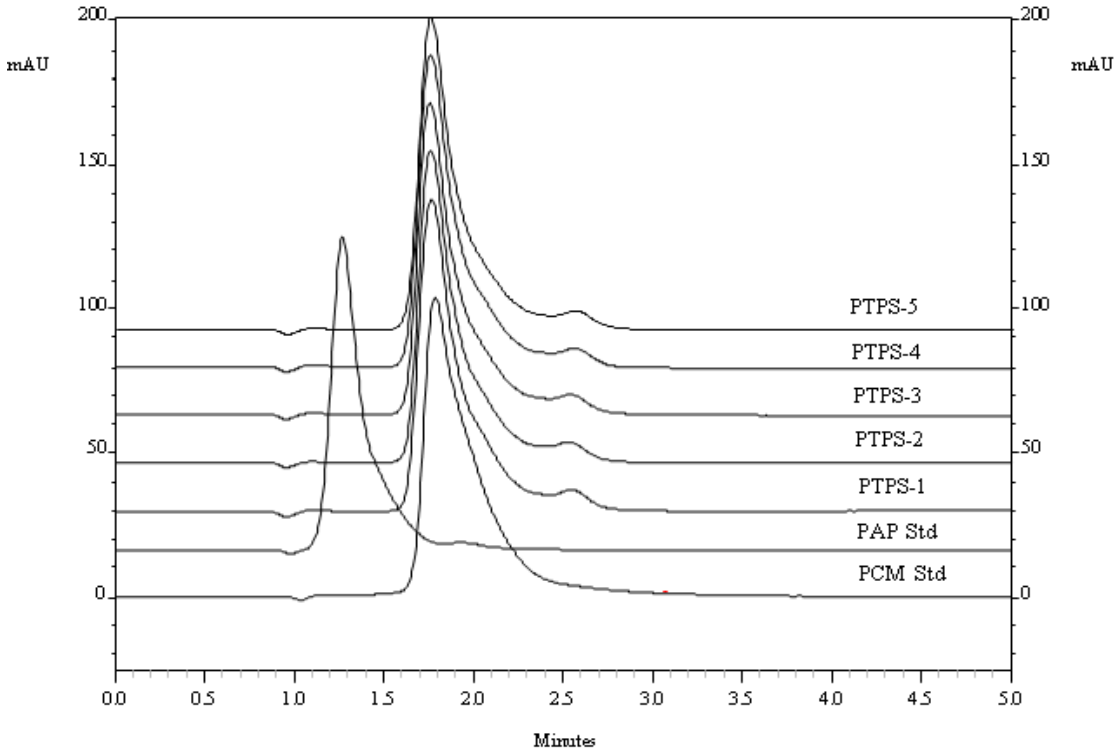

Fig. 7: HPL Chromatogram of PCM tabs Patent Store Samples for PAP Profiling.

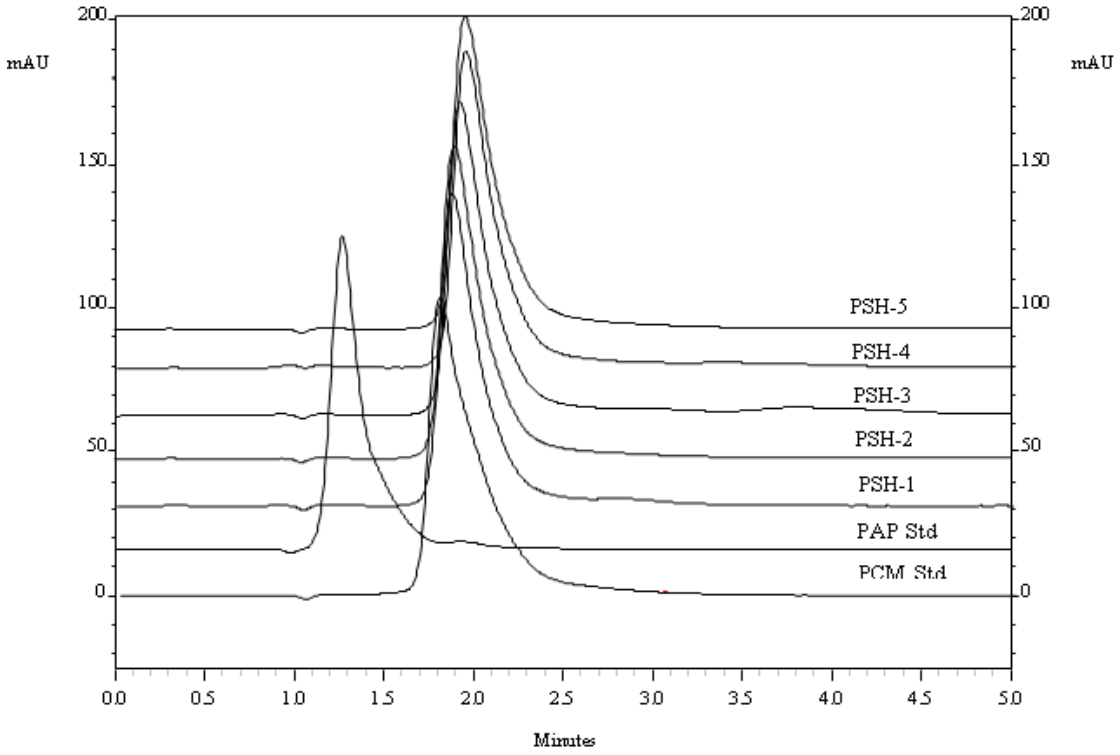

Fig. 8: HPL Chromatogram of PCM Syrup Hospital Samples for PAP Profiling.

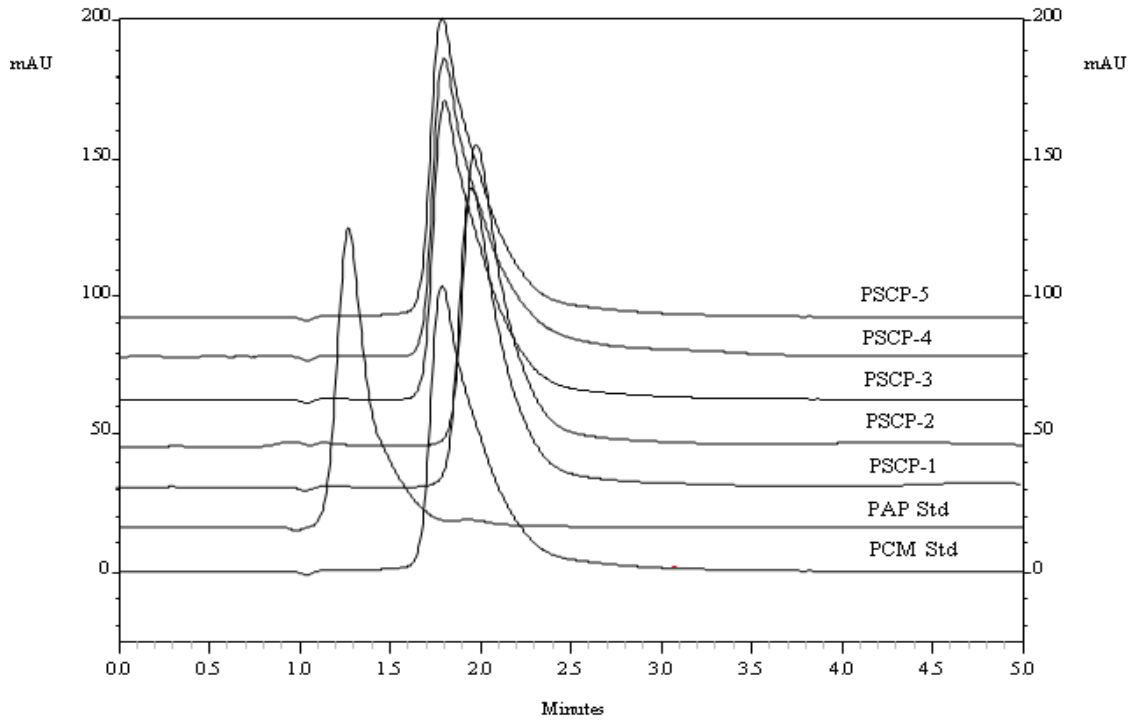

Fig. 9: HPL Chromatogram of PCM Syrup Community Pharmacy Samples for PAP Profiling.

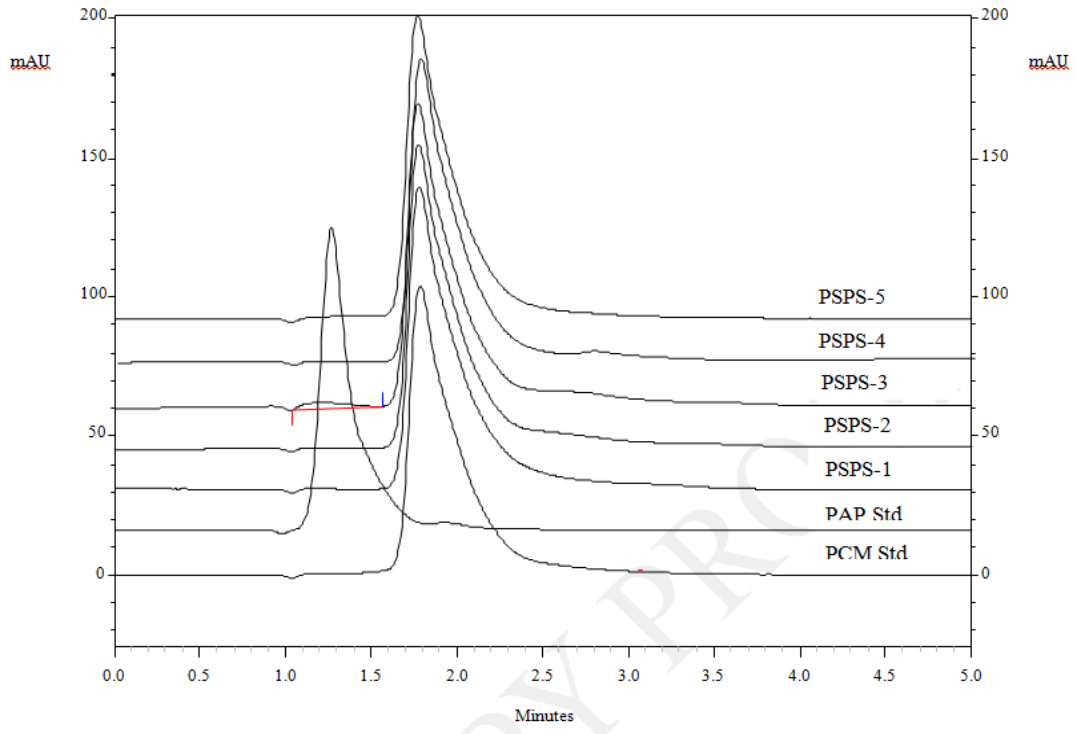

Fig. 10: HPL Chromatogram of PCM Syrup Patent Store Samples for PAP Profiling.

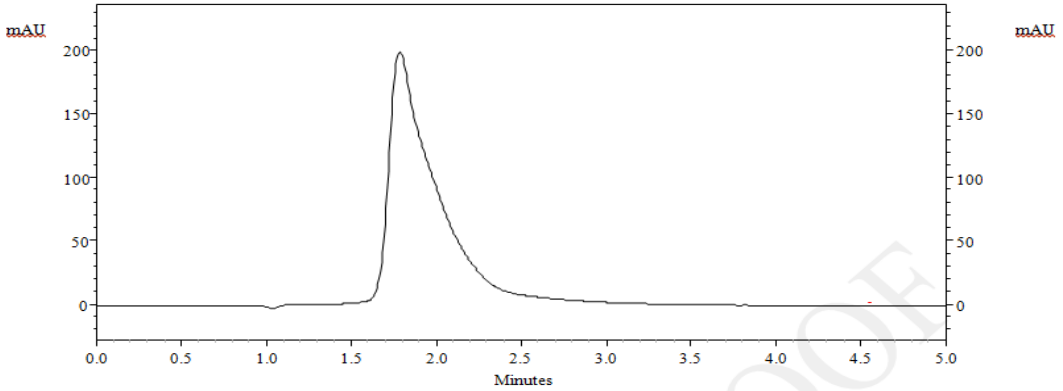

Fig. 11: PCM STD HPL Chromatogram.

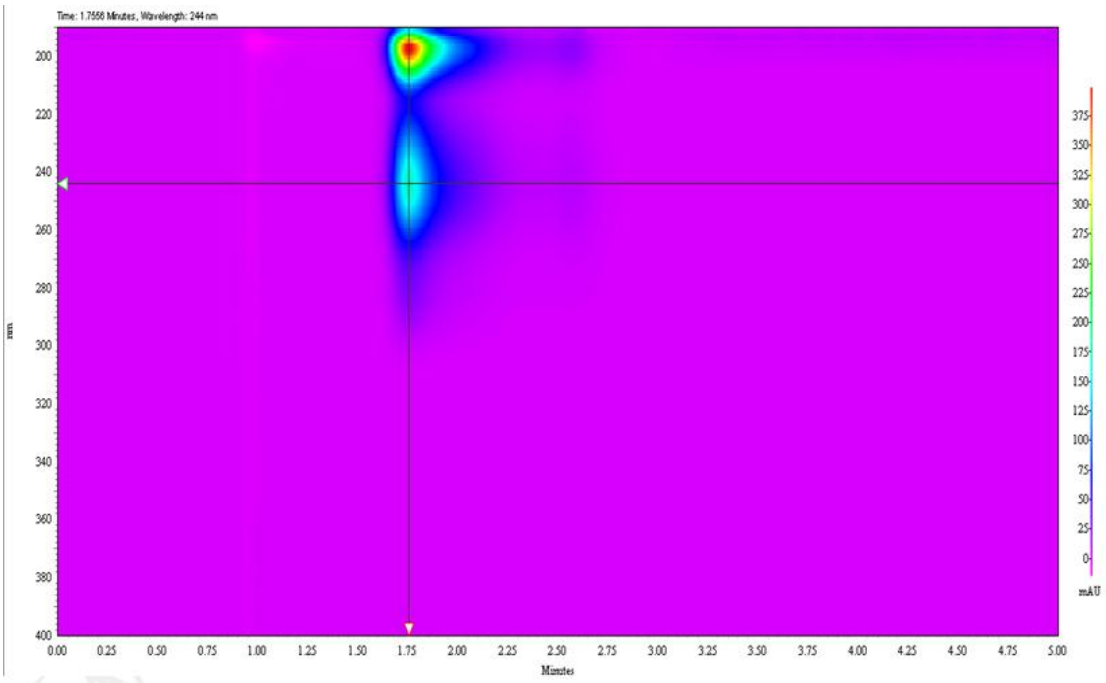

Fig. 12: Simulated TLC for PCM STD.

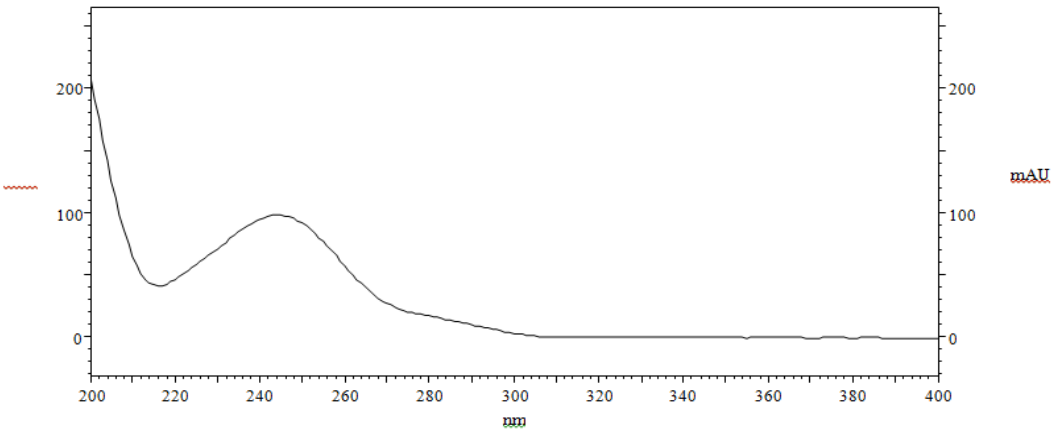

Fig. 13: PCM STD UV Spectrum; lambda max 244nm.

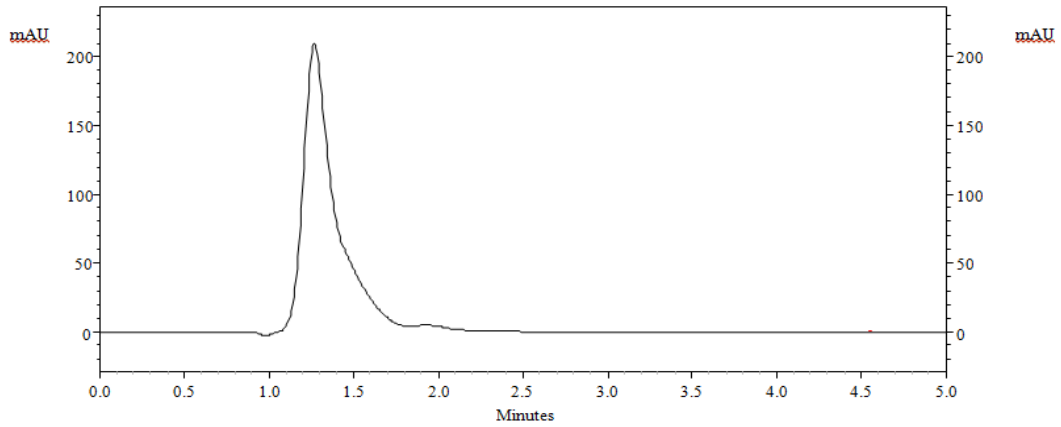

Fig. 14: HPL Chromatogram of PAP STD.

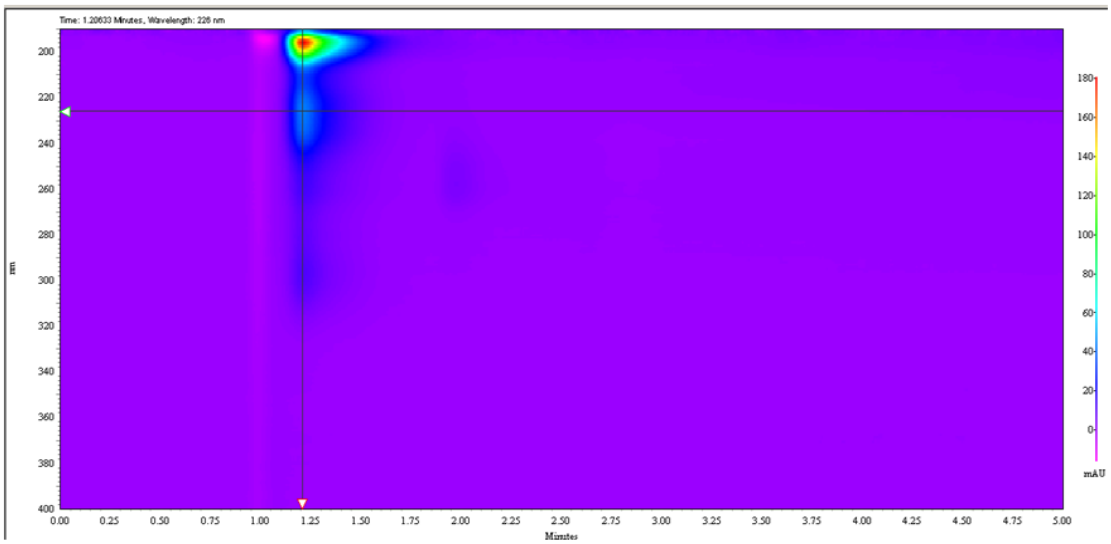

Fig. 15: Simulated TLC for PAP STD using DAD

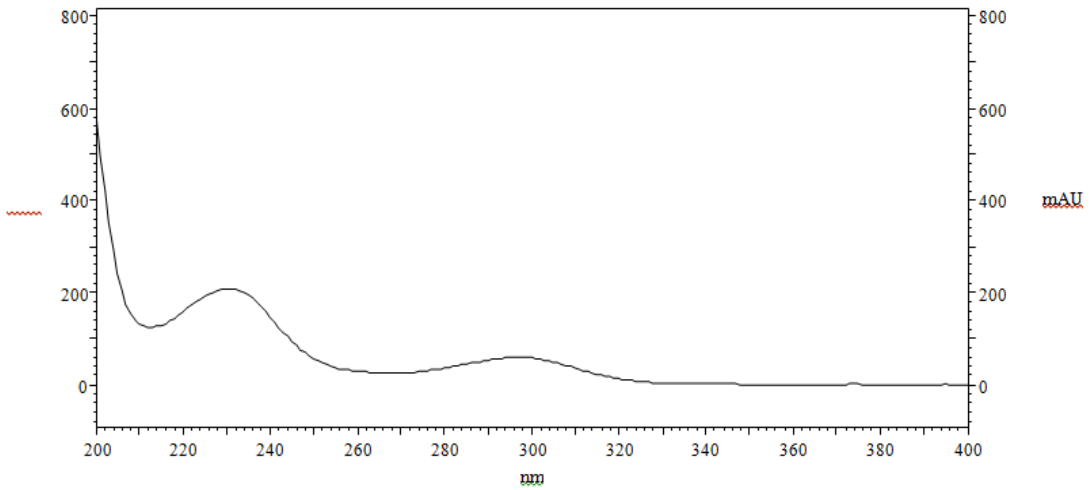

Fig. 16: PAP STD UV Spectrum; lambda max 231nm.

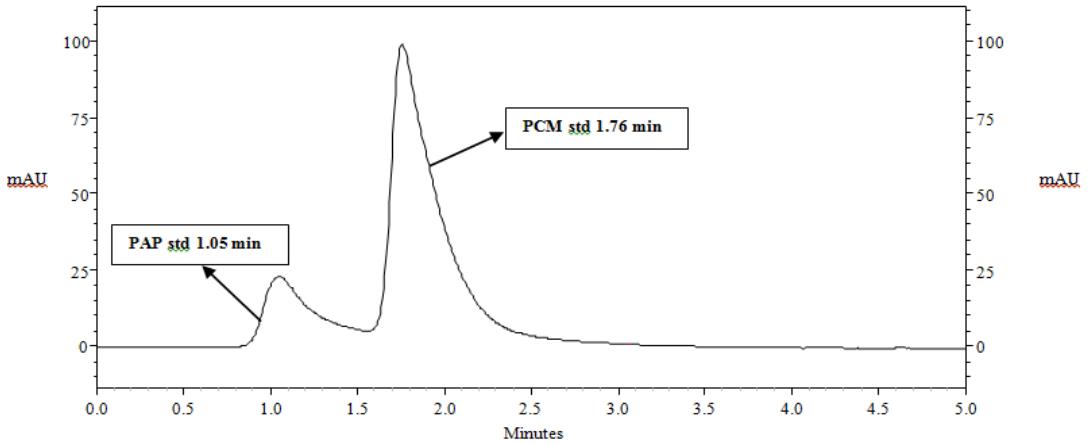

Fig. 17: HPL Chromatogram of Mixed PCM and PAP Standards 125 µg/ml each.

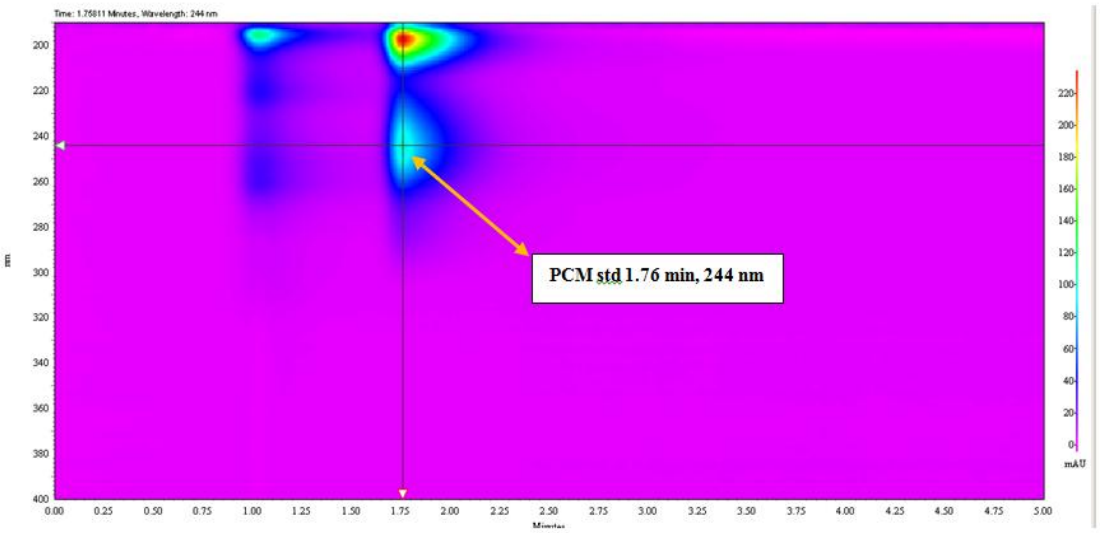

Fig. 18: Simulated TLC for mixed PCM and PAP STDs at PCM wavelength.

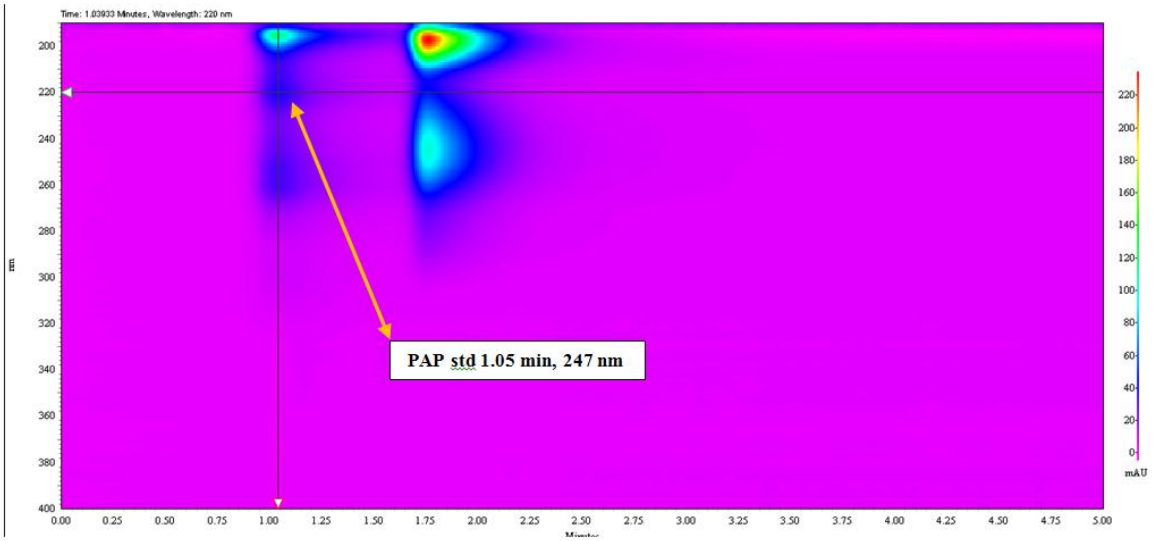

Fig. 19: Simulated TLC for mixed PCM and PAP STDs at PAP wavelength.

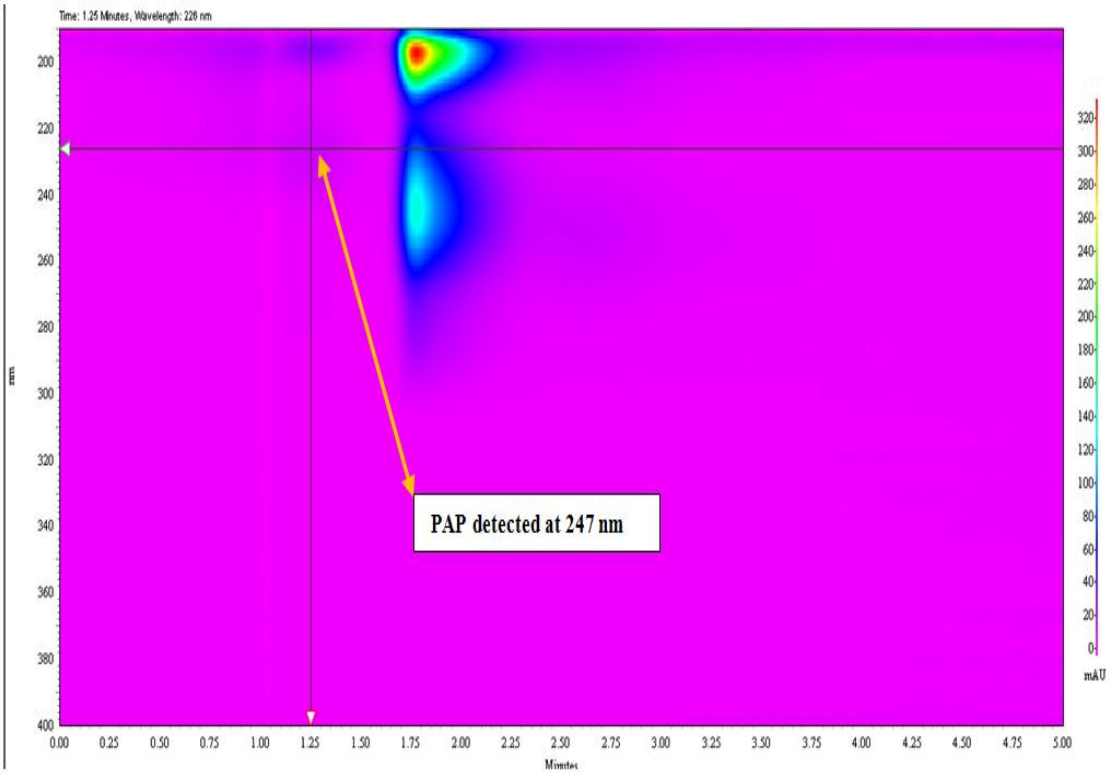

Fig. 20: PSPS 3 Positive Result at PAP wavelength.

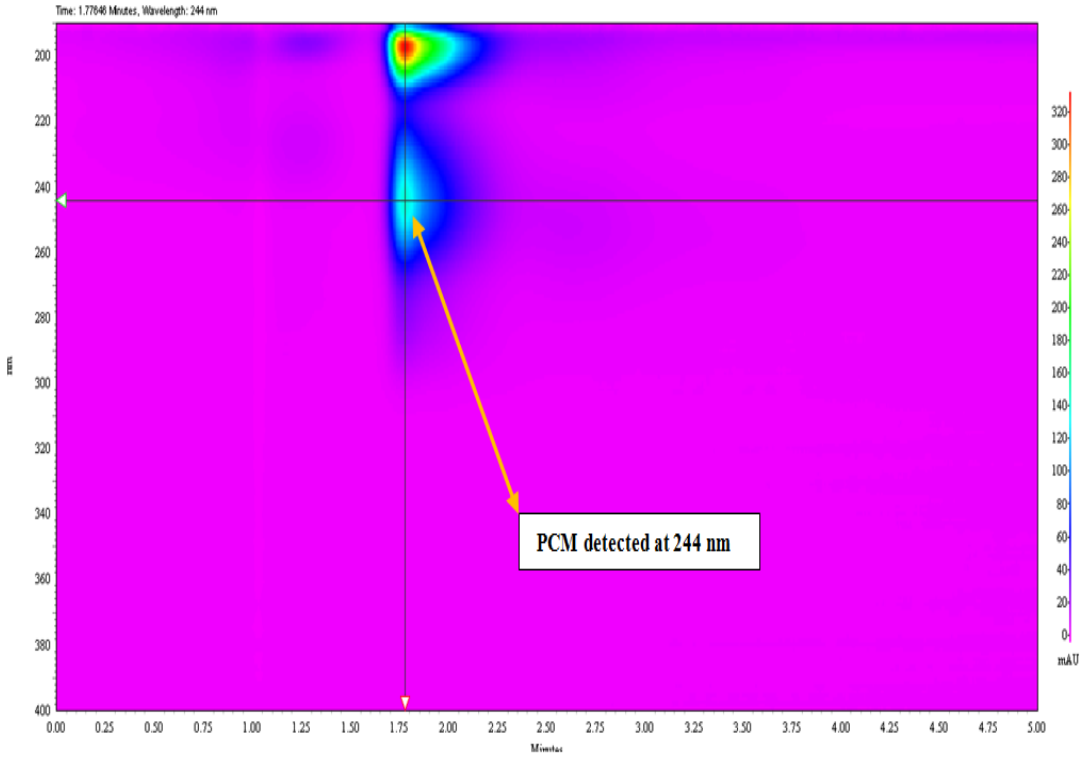

Fig. 21: PSPS 3 Positive Result at PCM wavelength.

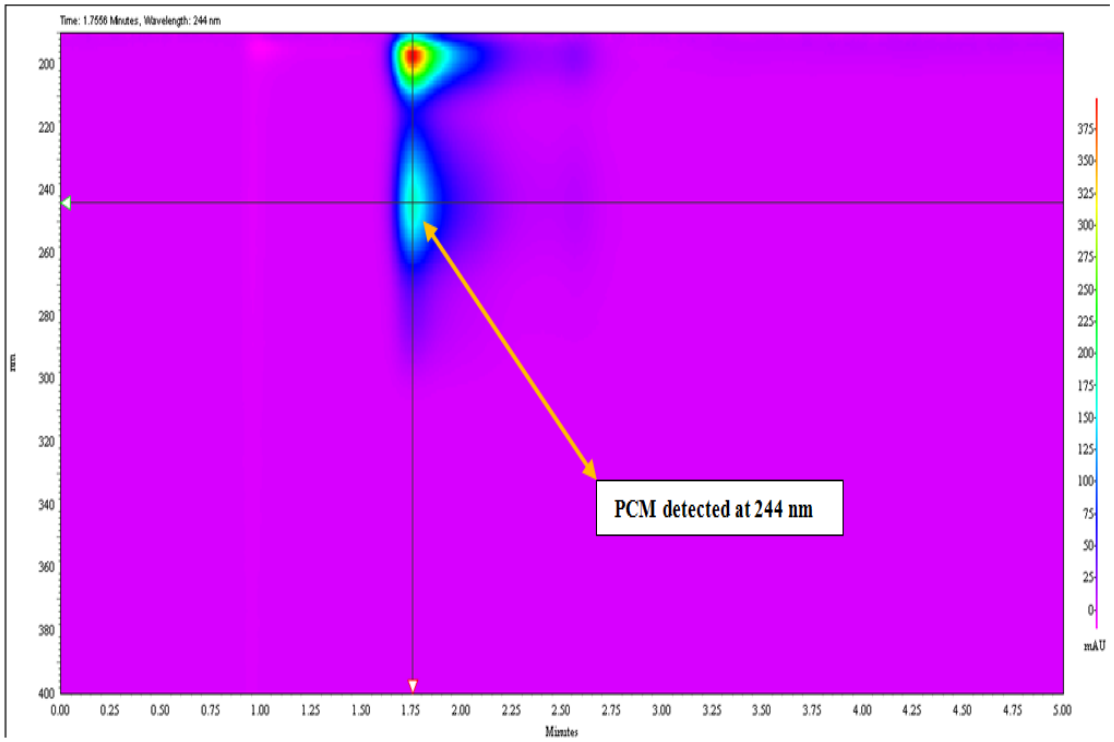

Fig. 22: Negative Result at PCM wavelength.

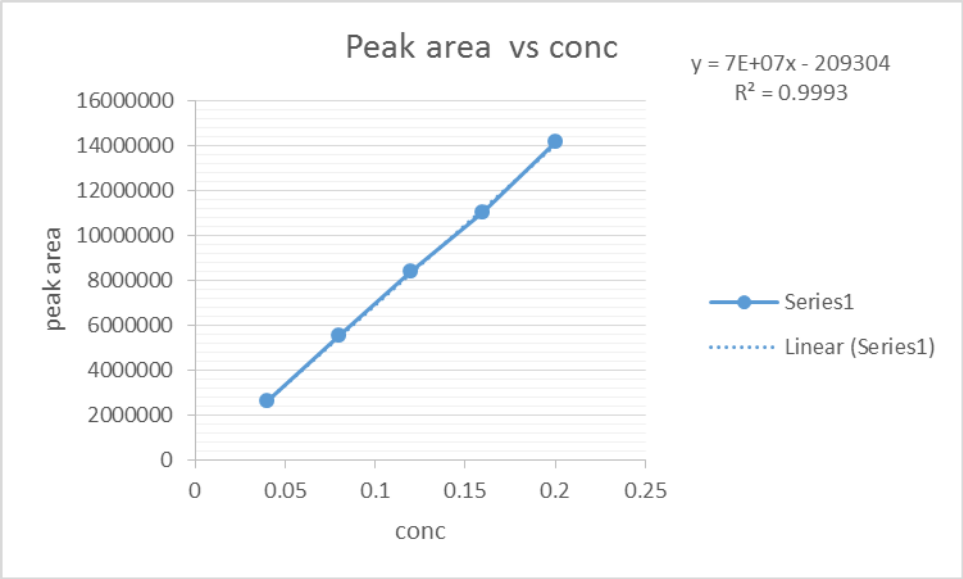

Fig. 23: PAP Calibration Curve using HPLC.

With regards to para-chloroacetanilide all the examined paracetamol dosage forms have shown no peaks corresponding to para-chloroacetanilideon the chromatograms. The chromatograms are shown below:

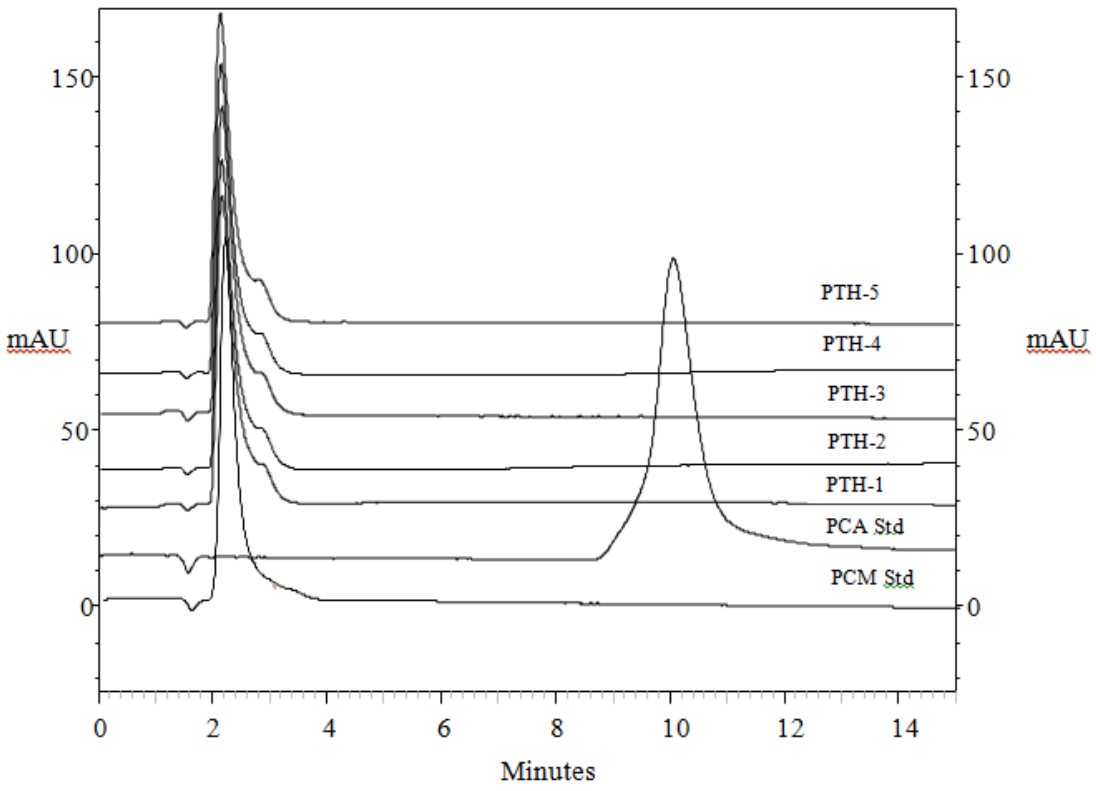

Fig. 24: HPL Chromatogram of PCM tabs Hospital Samples for PCA Profiling.

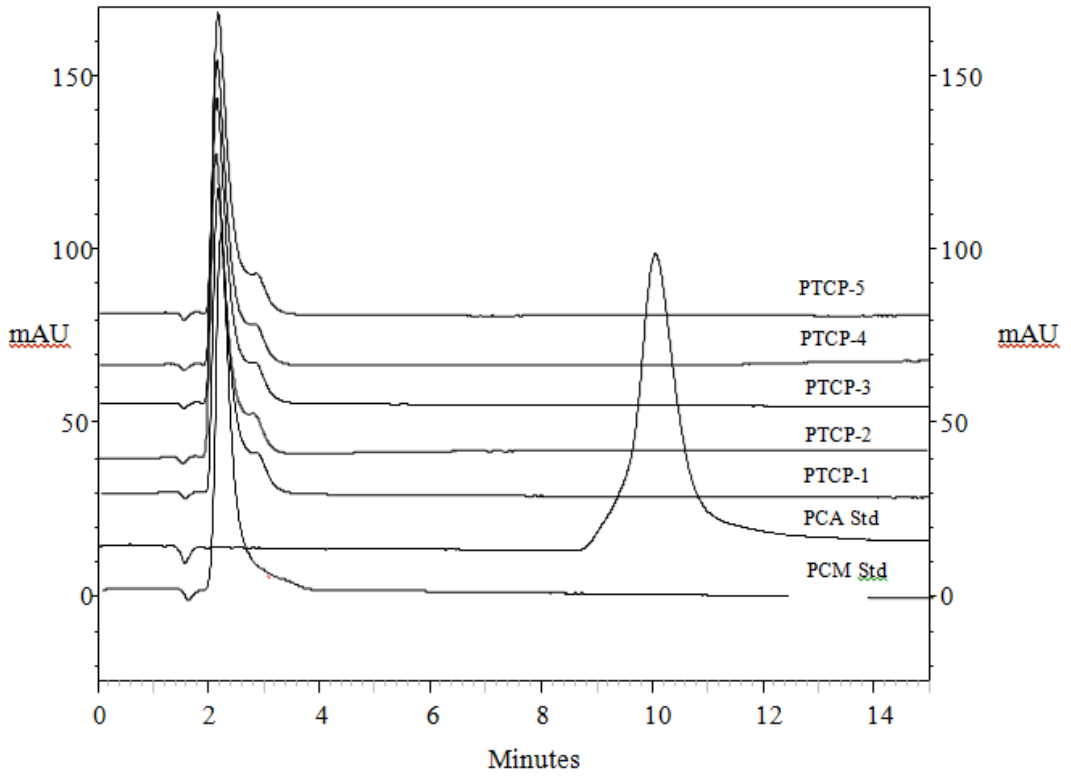

Fig. 25: HPL Chromatogram of PCM Tabs Community Pharmacy Samples for PCA Profiling.

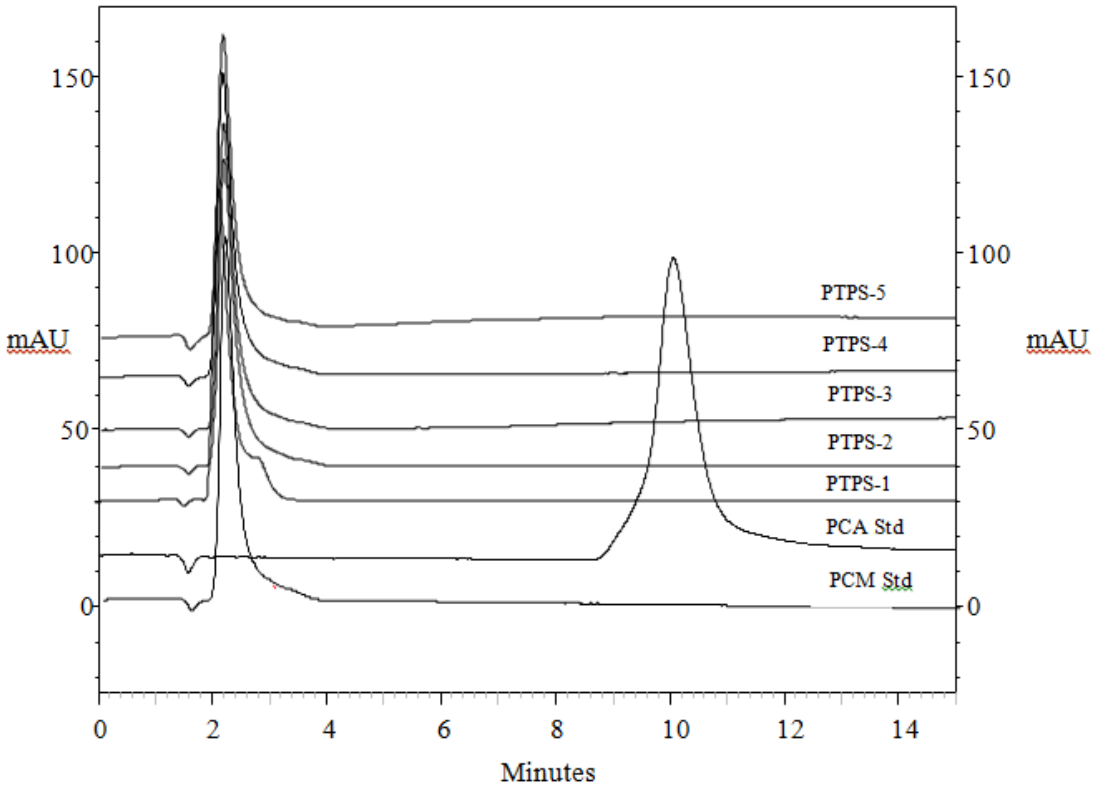

Fig. 26: HPL Chromatogram of PCM Tabs Patent Store Samples for PCA Profiling

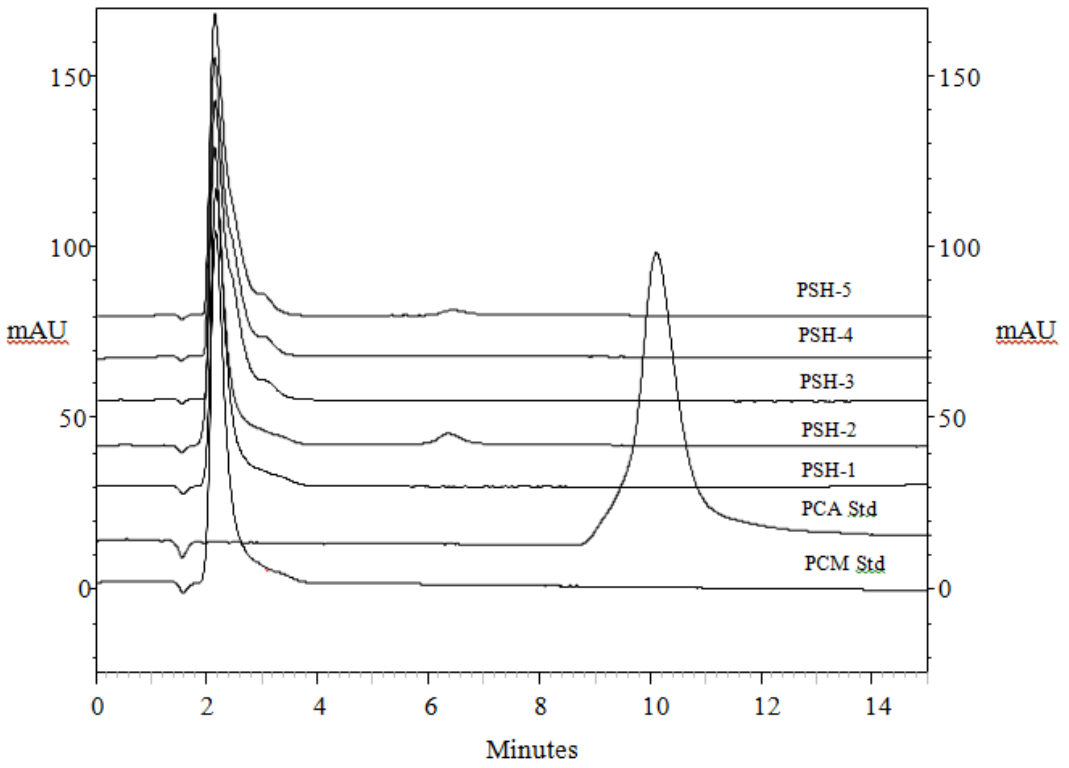

Fig. 27: HPL Chromatogram of PCM Syrup Hospital Samples for PCA Profiling.

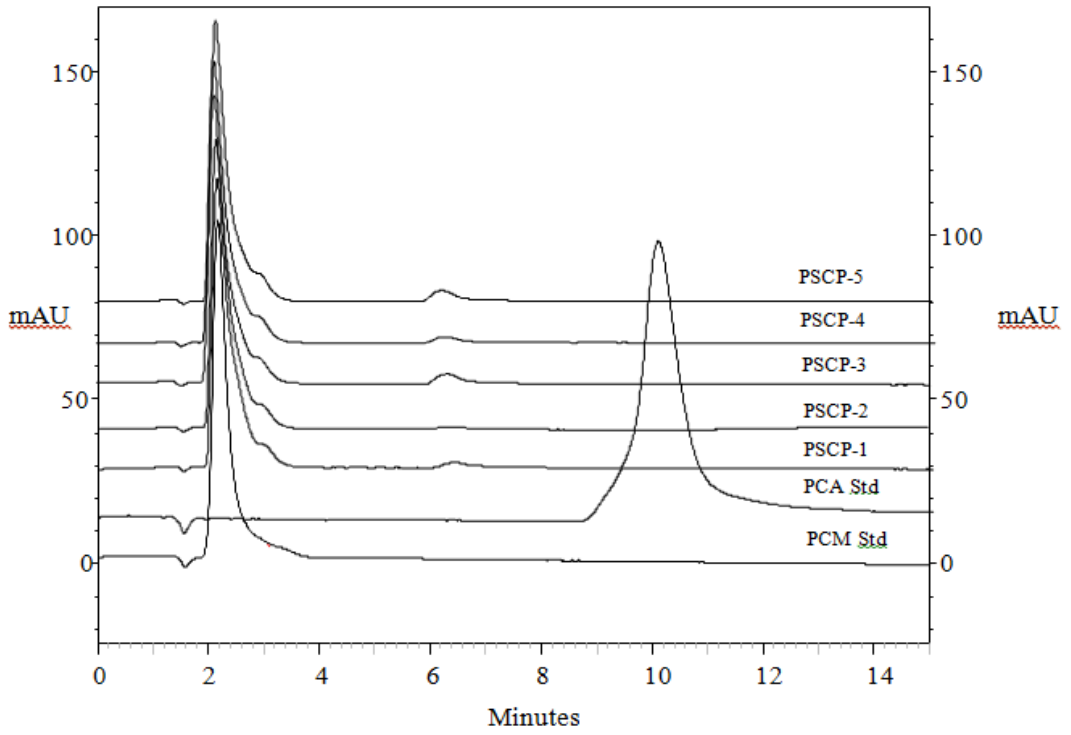

Fig. 28: HPL Chromatogram of PCM Syrup Community Pharmacy Samples for PCA Profiling.

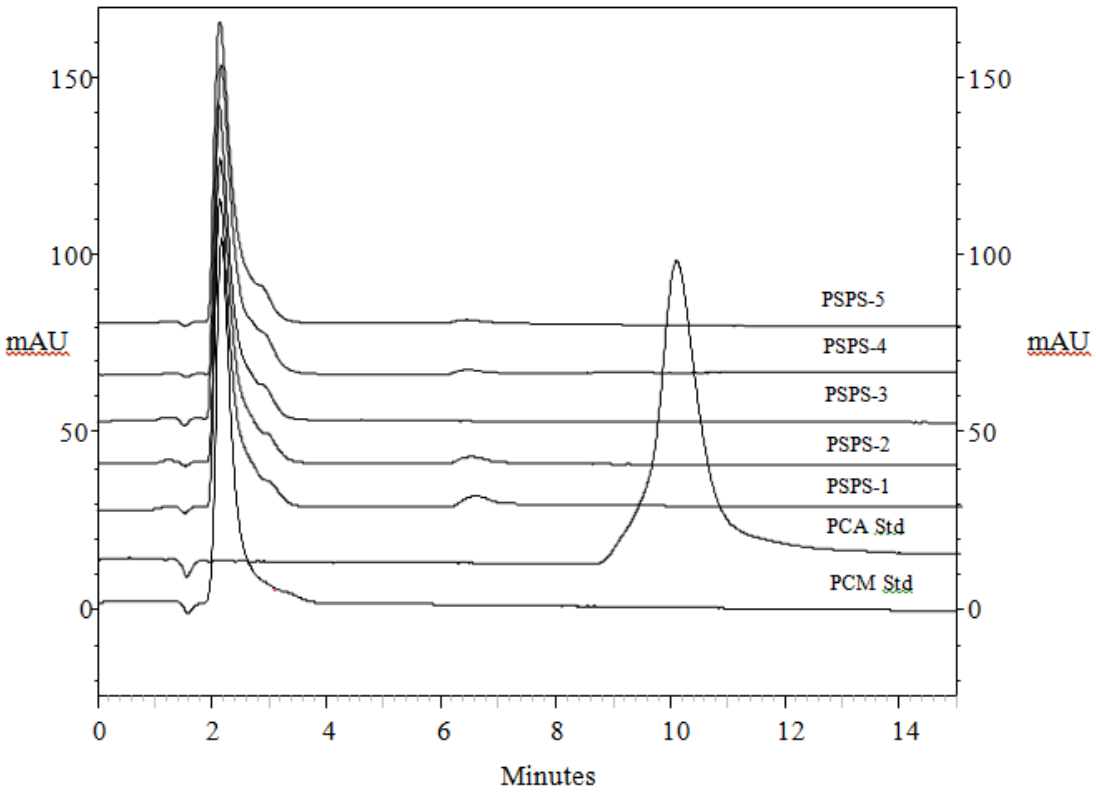

Fig. 29: HPL Chromatogram of PCM Syrup Patent Store Samples for PCA Profiling.

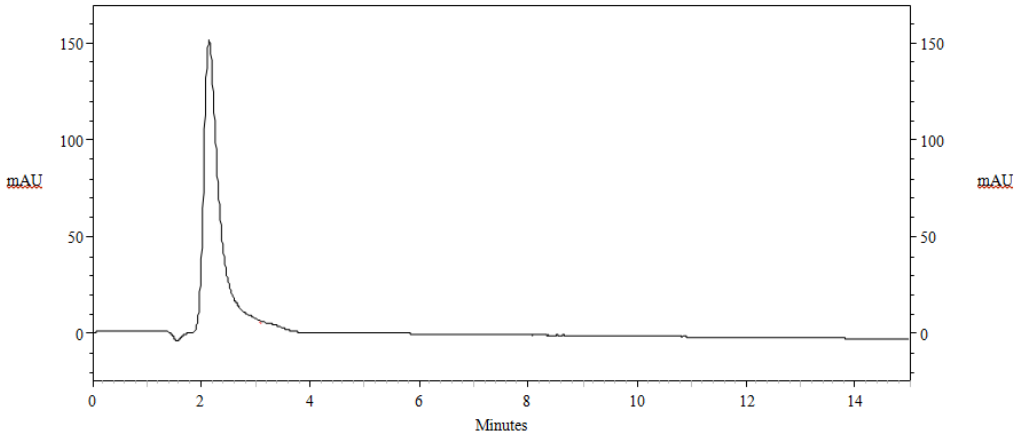

Fig. 30: HPL Chromatogram of PCM STD.

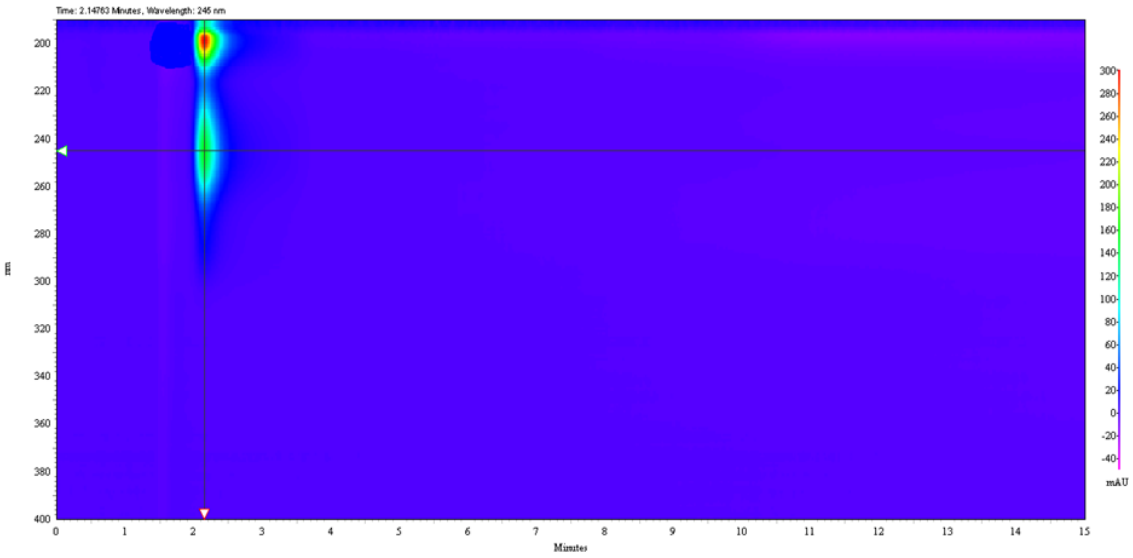

Fig. 31: Simulated TLC of PCM STD using DAD

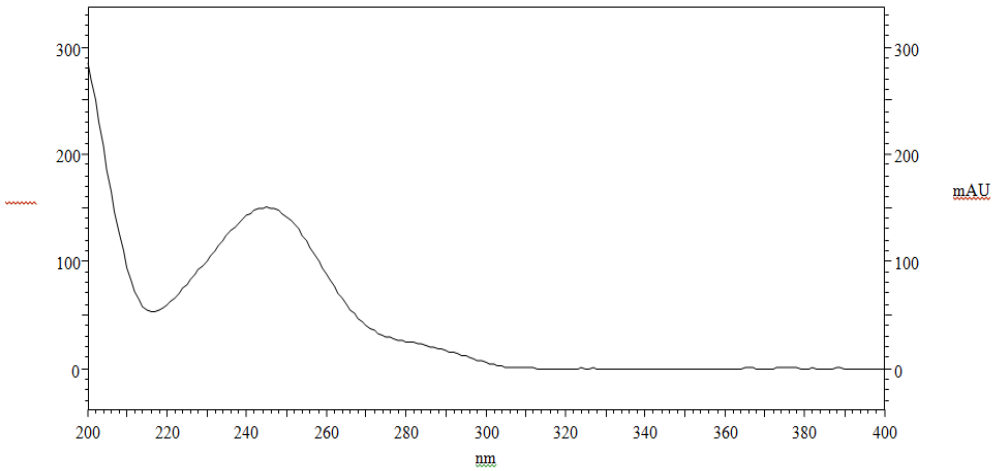

Fig. 32: PCM STD UV Spectrum; lambda max 244nm.

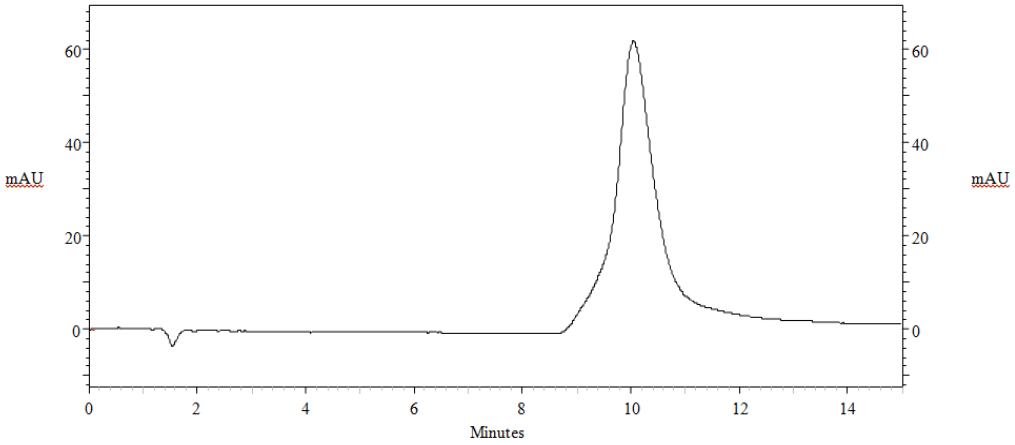

Fig. 33: HPL Chromatogram of PCA STD

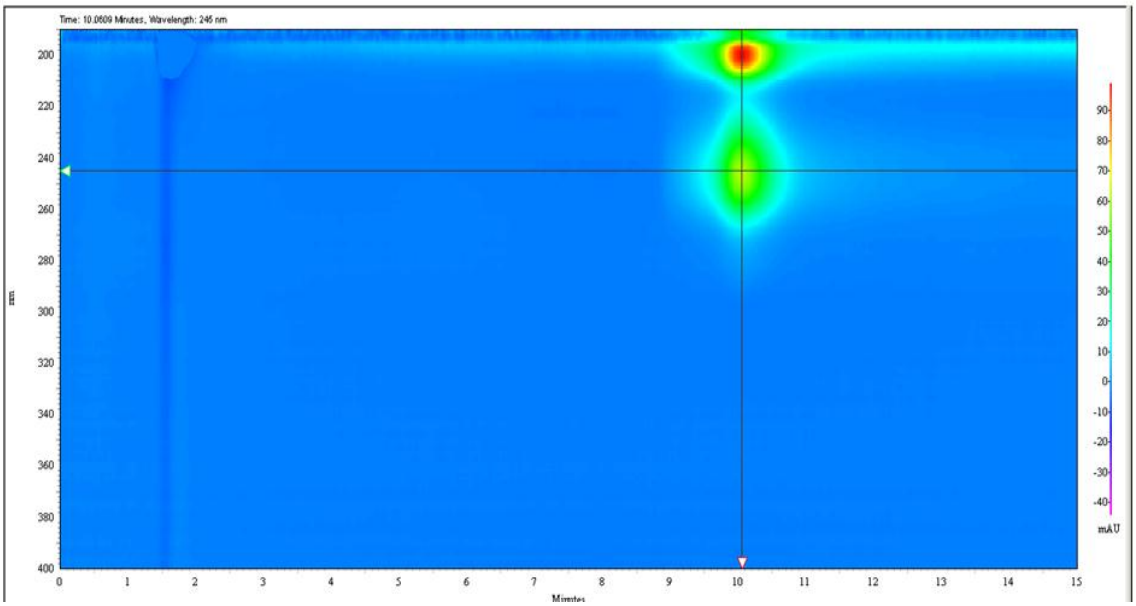

Fig. 34: Simulated TLC of PCA std using DAD.

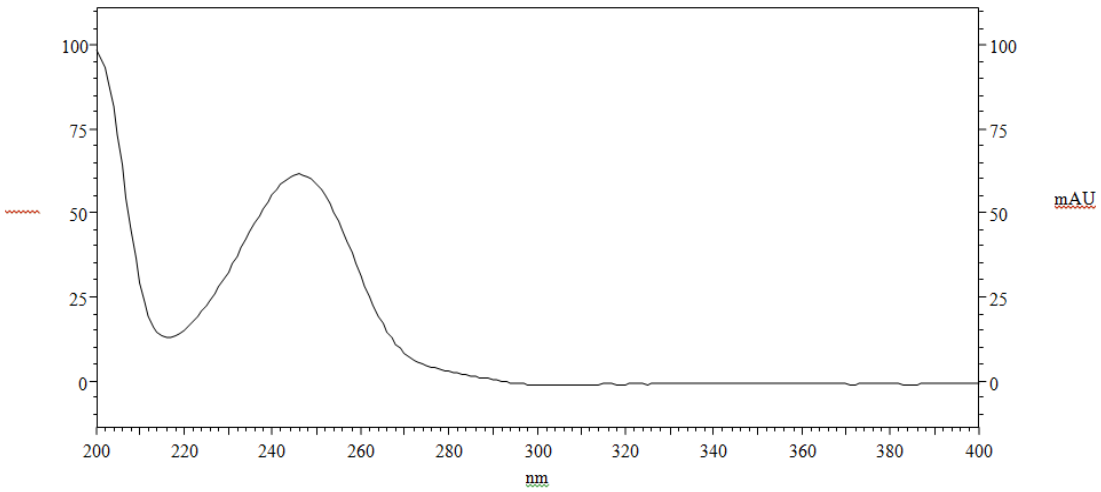

Fig. 35: PCA STD UV Spectrum; lambda max 247nm.

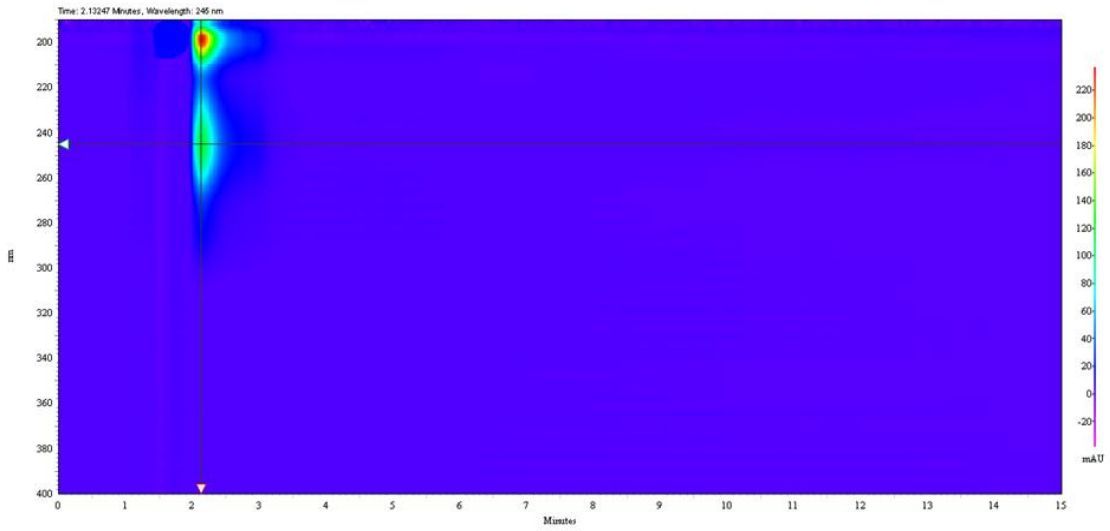

Fig. 36: PCM Sample for PCA Assay Negative Result.

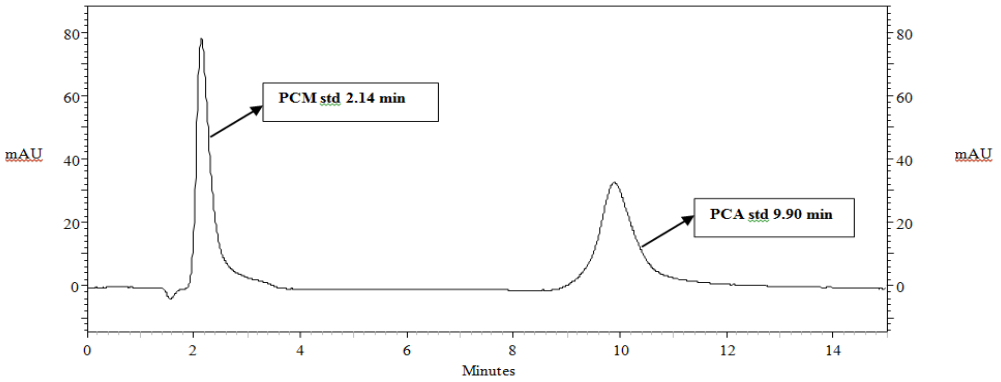

Fig. 37: HPL Chromatogram of Mixed PCM and PCA STDs 0.02 mg/ml each.

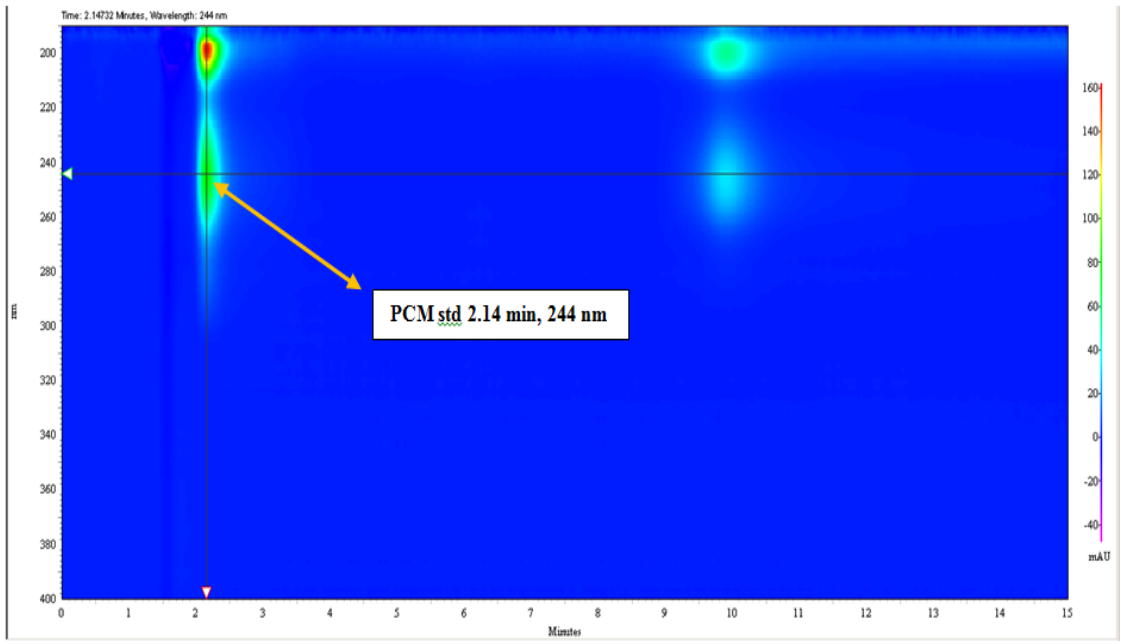

Fig. 38: Simulated TLC of Mixed PCM AND PCA STDs AT PCM Wavelength.

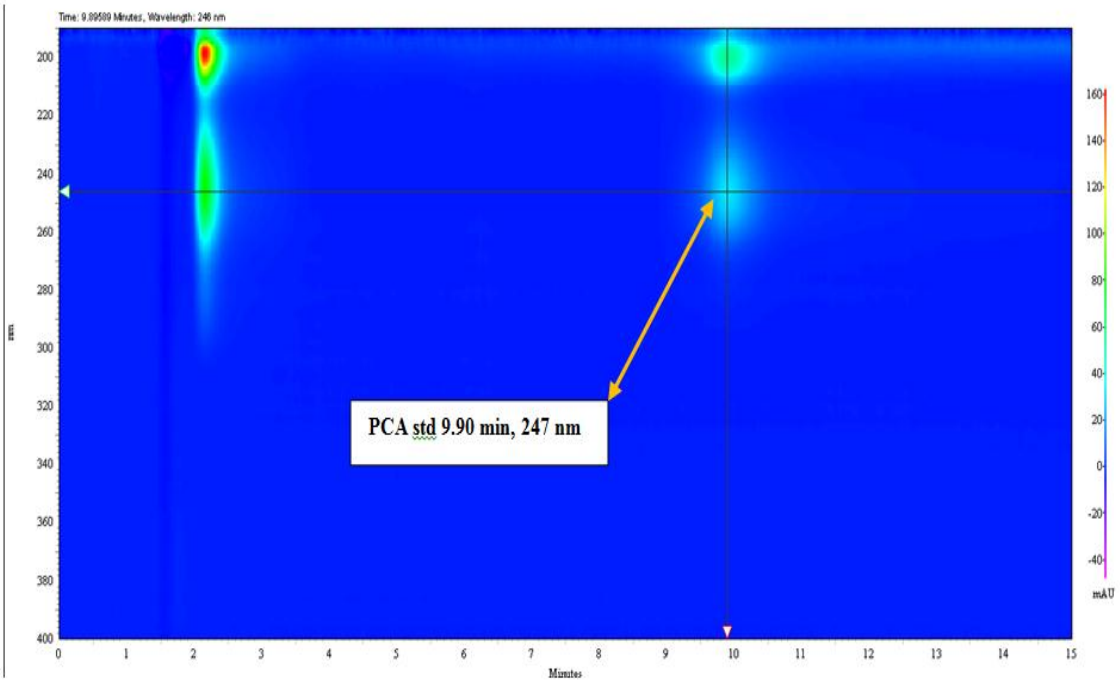

Fig. 39: Simulated TLC of Mixed PCM AND PCA STDs AT PCA Wavelength.

All the paracetamol samples used in the study showed no spots corresponding to the two impurities in question; PAP and PCA, thus the samples have passed the impurity test of pharmacopoeia.

HPLC is an advanced technique which can be employed in impurity profiling of drugs. Both qualitative and quantitative analysis can be carried out and it is able detect minute amount of impurity in a given sample.[19]

Using the HPLC method only patent store paracetamol syrup sample showed a peak corresponding to the impurity PAP, and as shown in the results it below the limit set by the pharmacopoeia.

HPLC techniques have been employed previously to determine paracetamol alone, in combination with other drugs and also its related substances otherwise known as impurities.[8,20,21]

The findings from the current study are similar to those reported in literature however there is variations in relation to retention time of the components which could be attributed to difference in solvent system used, column and other chromatographic conditions.

Despite the limitations attributed to the TLC method, it can still be employed when situations warrant as the case may be since the findings from the two methods are comparable and also as reported by Payka et al 2013.[22]

CONCLUSION

1. TLC and HPLC methods were successfully used to detect impurities in paracetamol samples.
2. Neither PAP nor PCA spot was detected using the TLC impurity profiling method of paracetamol samples.
3. One sample of paracetamol syrup presented with a peak that corresponds to PAP and upon quantification the amount was below the limit stated by the pharmacopoeia thus the sample was accepted. None of the samples showed peak corresponding to PCA using the HPLC method.

ACKNOWLEDGEMENT

We wish to express our appreciation to all students who helped in carrying out this study.

REFERENCES

1. Singh A., Afreen A., Singh D. P. and Kumar R. “A Review on Pharmaceutical Impurities and Their Importance”. World J Pharm Pharm Sci., 2007; 6(10): 1337-1354.
2. Saibaba S. V., Kumar M. S. and Ramu B. “Pharmaceutical Impurities and Their Characterization: A Review”. Eur J Pharm Med Res., 2016; 3(5): 190-196.
3. Akay C., Tuncer I., Sayal A., Aydin A., Zkan Y and Gul H. "Rapid and Simultaneous Determination of Acetylsalicylic Acid, Paracetamol, And Their Degradation and Toxic Impurity Products by HPLC in Pharmaceutical Dosage Forms". *Turk J Med Sci.*, 2008; 38(2): 167-173.
4. Kasture A.V., Mahadik K.R., Wadodkar S. G. and More H. N. “Impurities in Pharmaceutical Substances and Limit Tests”. *Pharm Anal*, 2008; 1: 6-9.
5. Bartos D. and Gorog S. "Recent advances in the impurity profiling of drugs". *Current Pharm Anal*, 2008; 4(4): 215-230.
6. Page C. and Curtis M. Integrated pharmacology, 3rd Edition, Mosby Elsevier, 2006; 334-336.
7. Sweetman S. C., (ed.). Martindale, 36th Edition. The Pharmaceutical Press, London, 2009; 20-21.

8. Nageswara Rao R. and A. Narasaraju A., (2006). “Rapid Separation and Determination of Process-Related Substances of Paracetamol Using Reversed-Phase HPLC with Photo Diode Array as a Detector”. *Anal Sci.*, 2006; 22: 287-292.
9. USP 40 NF 35, United States Pharmacopeia. U.S. Pharmacopeial Convention, Inc., Rockville, MD, 2017.
10. British Pharmacopoeia, Vol. I-IV. Published by the department of health, London, 2018; 1889-1898, 4548-4552.
11. Espinosa M., Ruiz A., Sanchez F. and Bosch C."Determination of Paracetamol: Historical Evolution". *J Pharm Biomed Anal*, 2006; 42(3): 291-321.
12. Thomas S.H.L. "Paracetamol (Acetaminophen) Poisoning". *Pharmacol Therap*, 1993; 60: 91-120.
13. Harmon R."Pyruvate reduces 4-aminophenol in vitro toxicity". *Toxicol Appl Pharmacol*, 2006; 213: 179-186.
14. Chen G, Ye J, Bao H and Yang P. "Determination of the Rate Constants and Activation Energy of Acetaminophen Hydrolysis Bb Capillary Electrophoresis". *J Pharm Biomed Anal*, 2002; 29(5): 843-850.
15. Bloomfield M. and Talanta S. "A Sensitive and Rapid Assay for 4-Aminophenol in Paracetamol Drug and Tablet Formulation, by Flow Injection Analysis with Spectrophotometric Detection". *J Pharm Biomed Sci*, 2002; 58: 1301-1310.
16. Osterhuis B. "Specific interactions of chloroacetanilide herbicides with human ABC transporter proteins". *Toxicol*, 2008; 248: 45-51.
17. Crevar M., Ivkovic B., Vladimirov S., Kuntica V. and Vujic Z. “Statistical Optimization of Reverse Phase High Performance Liquid Chromatography for the Analysis of Caffeine Paracetamol and its Degradation Product p-aminophenol”. *Acta Chim Slov*, 2008; 55: 665–670.
18. Ali M. S., Rafiudin S., Ghori M. and Kahtri A.R. “Simultaneous Determination of paracetamol, Chlorzoxazone and Related Impurities 4-aminophenol, 4′-chloroacetanilide and p-chlorophenol in pharmaceutical preparations using high-performance liquid chromatography”. *J AOA CInt*, 2007; 90(1): 82-93.
19. Badyal P. N., Sharma C., Kaur N., Shankar R., Pandey A. and Rawal R. K. “Analytical Techniques in Simultaneous Estimation: An Overview”. Austin J Anal Pharm Chem, 2015; 2(2): 1-14.

20. Ca linescu O., Badea I. A., Vladescu L., Meltzer V. and Pincu E. "HPLC Separation of Acetaminophen and its Impurities Using a Mixed-mode Reversed-Phase/Cation Exchange Stationary Phase". *J Chromatogr Sci*, 2012; 50: 335–342.
21. Raju K. N., Sunitha T., Krishnaja V. and Babu I. S. "Development and Validation of RP-HPLC method for the Simultaneous estimation of Meloxicam and Paracetamol". Int. J. Drug Dev. & Res., 2013; 5(4): 280-285.
22. Pyka A., Budzisz M. and Dolowy M. "Validation Thin Layer Chromatography for the Determination of Acetaminophen in Tablets and Comparison with a Pharmacopeial Method". *Biomed ResInt*, 2013; 1-10.