

**Dual-mode gradient HPLC and TLC densitometry methods for
the simultaneous determination of paracetamol and methionine
in the presence of paracetamol impurities**

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Abstract

Background: Paracetamol is one of the most widely analgesic and antipyretic drugs recently integrated into the supportive therapy of COVID-19. The pharmaceuticals containing methionine with paracetamol may contribute to avoid hepatotoxicity and eventual paracetamol overdose-dependent death.

Objective: The current work purposes to develop and validate two chromatographic methods for the simultaneous determination of methionine and paracetamol in presence of two paracetamol impurities (4-nitrophenol and 4-aminophenol).

Methods: Two chromatographic methods were established and validated according to the International Conference on Harmonization guidelines. The first one was a RP-HPLC/UV method based on applying a “dual-mode” gradient elution. The separation was realized via varying both the composition of the ternary mobile phase (acetonitrile–methanol–water) and its flow rate. This strategy enabled a relatively rapid analysis with a satisfactory resolution, although the investigated compounds exhibit a significant difference in lipophilicity. The second one relied on TLC- densitometry, where the optimum separation was realized using a quaternary mobile phase system composed of butanol–dioxane–toluene–methanol (8: 2.5: 3.5: 0.3, by volume). Both methods were monitored at 220 nm.

Results: The developed methods were proven to be robust, accurate, specific, and appropriate for the routine analysis of paracetamol in its pure form or in pharmaceutical formulations with methionine in quality control laboratories.

Conclusions: The corresponding methods are suitable to determine methionine and paracetamol in the presence of paracetamol impurities.

Highlights: The study achieves the analysis of methionine and paracetamol in the presence of paracetamol impurities via the application of HPLC and TLC- densitometry methods.

Keywords:

Dual-mode gradient; HPLC; Methionine; Paracetamol; TLC.

Non-steroidal anti-inflammatory drugs are among the most commonly prescribed medicaments worldwide to treat a diversity of pain-related conditions (1). Among them, paracetamol (PC) is often the analgesic or antipyretic of choice, especially in the elderly and in patients in whom salicylates or other NSAIDs are contra-indicated (2). Such patients include asthmatics, those with a history of peptic ulcer, and children. Although paracetamol is a safe and well-tolerated drug at the recommended doses, it is the most commonly overdosed drug inducing life-threatening toxicity and death (3). PC has been implemented recently into the supportive therapy of COVID-19, but PC may induce hepatotoxicity at high doses (4), (5). Methionine (MT) is considered an essential amino acid and implicated in glutathione biosynthesis. Though, in case of paracetamol overdoses, it serves as an antidote via increasing glutathione levels and hence preventing significant tissue damage (6) and may contribute to avoiding hepatotoxicity and eventual overdose-dependent death (7), (8). A combination of methionine with paracetamol reduces the acute toxicity of paracetamol by 50%. They are marketed in combination as tablets to treat fever, headache, and pain (9).

4-Aminophenol (AP) was considered as the chief co-existing impurity of paracetamol in pharmaceutical preparations resulting from either degradation or synthesis (10), (11). As AP is a pharmacologically active substance owning teratogenic and nephrotoxic effects, consequently its concentration should be strictly tracked (11). 4-Nitrophenol (NP) is the precursor of the AP and considered a potential paracetamol impurity (12).

Several methods have been reported for the assay of PC (13–18) and a recent review mentioned the different analytical techniques implicated in PC analysis (19). Few methods have been described the assay of MT in different matrices (20–22) and in pharmaceuticals (23)

and the combination of both MT and PC (24), (25). However, to the best of our knowledge, the literature survey did not reveal any method for the determination of PC and MT mix in the presence of PC impurities.

The current work intends to develop and validate robust, sensitive, and selective chromatographic methods for the simultaneous determination of MT and PC in the presence of paracetamol impurities in pharmaceuticals. A reversed-phase high-performance liquid chromatography coupled to UV-detection (RP-HPLC/UV) and thin-layer chromatography (TLC) - densitometry methods were developed and optimized for the rapid separation of the investigated compounds which displayed significant different lipophilic characters.

The obtained results were statistically analyzed and the methods were validated as per the International Conference on Harmonization (ICH) guidelines (26).

Experimental

Materials and Reagents

Paracetamol and DL-methionine working standards were kindly provided by Hikma Pharmaceuticals Co, 6th October, Giza, Egypt; their purities were reported to be 99.12 ± 1.37 and $99.65 \pm 1.26\%$, respectively. Hepamol[®] Tablets manufactured by Hikma Pharmaceuticals Co., labeled to contain 100 mg DL-methionine and 500 mg paracetamol per tablet, were purchased from the local market. AP (Acetaminophen RCK) and NP (Acetaminophen RCF) (27) were purchased from Sigma-Aldrich. Acetonitrile and methanol were HPLC-grade (Fisher-Scientific, UK). Butanol, dioxane and toluene were of analytical grade (Adwic Co, Egypt).

Instrumentation and Chromatographic Conditions

(a) HPLC method.—HPLC/UV (Agilent 1260 infinity) system composed of a Quaternary pump (model G 711A, Quat pump VL 1260, Waldbronn-Germany), a Rheodyne injector

(model 7225/7725I) assembled with 20 μ L injector loop (Rohnert Park, CA, USA) and an ultraviolet-visible (UV-Vis) multiple wavelength detector (model G7165 A, 1260 MWD). Data acquisition was performed using Open Lab CDS ChemStation[®] (version A.01.05) software. Mobile phase system composed of water, methanol, and acetonitrile and the dual-mode gradient elution was performed via programming both mobile phase flow rate and solvents ratios. The UV-Vis detector was set at 220 nm. The column used in chromatographic separation was Zorbax[®] SB C-8 column, 5 μ m, 150 mm \times 4.6 mm (Agilent Technologies, USA). The column was equilibrated with the mobile phase for 30 min. prior to injection. 20 μ L of each sample were injected into the analytical column. The separation was performed at room temperature under the specified chromatographic conditions as described in Table 1.

(b) TLC densitometry method.—TLC densitometer system consisted of Camag Linomat 5 autosampler equipped with Camag microsyringe 100 μ L. A Camag (Switzerland) TLC Scanner-3 densitometer model-3 supplied with WinCats[®] software version 1.4.2.8121. The spraying rate and scanning speed were 10 s/mL and 20 mm/s, respectively. The separation was achieved on TLC aluminum sheet silica gel 60 F₂₅₄ plates (10 \times 20 cm) (Merck, Germany). Samples were quantitatively spotted onto the TLC plates, by using Camag Linomat autosampler via 100 μ L microsyringe. The spotted bands were 15 mm from the bottom side edge of the plate, and 6 mm length, 10.5 mm spaced from each other. The optimum mobile phase composition used for the chromatographic separation was butanol–dioxane–toluene–methanol (8:2.5:3.5:0.3 by volume). The mobile phase ran about 8 cm. The plates were developed by ascending chromatography to 8 cm from the spotting line, in a chromatographic chamber saturated previously with the mobile phase at room temperature for 60 min. The plates were kept at room temperature for 30 min till dryness then the bands were scanned using a UV lamp which set at 220 nm in the absorption mode at a scanning speed of 20 mm/s for densitometric determination.

Validation Procedure

The chromatographic methods were developed and validated regarding linearity, accuracy, precision, specificity, limit of detection, limit of quantitation, robustness, and system suitability test.

(a) Linearity.—Stock standard solutions (1000 µg/mL) of each compound were prepared in 10% (v/v) aqueous methanol.

For the HPLC method, the working standards were prepared from these stock solutions by appropriate dilution with the mobile phase providing a final concentration range of 10–200 and 20–600 µg/mL for MT and PC, respectively. Each concentration was injected into HPLC in a triplicate. The calibration curves were obtained by plotting the concentration of each standard against the corresponding peak area using the linear regression method to establish the linearity.

For the TLC method, accurate volumes from each stock standard solution (1000 µg/mL) were spotted onto 10 × 20 cm TLC plates to provide 2–12 and 5–30 µg / spot for MT and PC, respectively by using Camag Linomat autosampler via 100 µL microsyringe. Calibration curves were constructed by plotting the peak area of each standard against their corresponding concentrations and the linear regression equations were achieved.

(b) Accuracy.—The accuracy was assessed by using at least nine determinations over a minimum of three different concentration levels which covered the specified range and expressed as the mean of the percent recoveries ± SD. In the TLC method, the concentrations were 2, 4, and 6 µg/spot and 6, 8, and 10 µg/spot for MT and PC, respectively. For the HPLC method, the concentrations tested were 20, 40, and 60 µg/mL for MT and 50, 75, and 100 µg/mL for PC. The above-mentioned procedures were realized for the determination of different concentrations where each concentration was calculated via its corresponding linear regression equation.

(c) *Precision*.—The inter-day precision was determined over three different concentration levels within the specified range of each standard and done in triplicate in three consecutive days. For the determination of repeatability, the intraday precision studies were conducted by analysis of three different concentration levels within the specified range of each standard and done in triplicate within the same day. For the HPLC method, the three tested concentration levels were 40, 80, 100 µg/mL, and 200, 300, 400 µg/mL for MT and PC, respectively. For the TLC method, the three evaluated concentrations were 4, 6, 10 µg/spot and 10, 20, 25 µg/spot for MT and PC, respectively. Different concentrations were determined according to the previously mentioned chromatographic procedures.

(d) *Specificity*.—The standard working solutions of each impurity (NP and AP) were prepared in 10% aqueous methanol at a concentration of 1000 µg/mL. The separation of the laboratory prepared mixes containing variable ratios of MT, PC, AP, and NP was realized using the above-mentioned chromatographic procedures. Equations of the linear regression of both MT and PC were used to evaluate the ability of each method to detect MT without the interference of PC and its impurities and to detect PC separately in presence of MT and PC impurities. Specificity (mean of percent recoveries ± SD) of recovery percent data of laboratory prepared mixes.

(e) *LOD and LOQ*.—The LOD and LOQ of both methods were calculated for MT and PC. Data obtained from the calibration curves were involved in the determination of LOD and LOQ using the following equations:

$$\text{LOD} = 3.3 * \text{SD of intercept} / \text{slope}$$

$$\text{LOQ} = 10 * \text{SD of intercept} / \text{slope}$$

(f) *Robustness and system suitability testing*.—Robustness was assessed in terms of RSD after application of small variations in the composition of the mobile phase and its saturation time for the TLC method and in the mobile phase composition, its flow rate, and detector wavelength

for the HPLC method. The system suitability testing parameters were assessed regarding tailing factor, selectivity factor, resolution, and theoretical plate number.

Application to Pharmaceuticals and the Standard Addition Technique

Twenty tablets were weighted to determine the mean weight then they were finely ground. The mean weight of the ground powder (equivalent to 100 mg of MT and 500 mg of PC) was accurately weighed out and dissolved via 15 min sonication into 200 mL of the same solvent as the standards. The solution was then filtered and quantitatively transferred into a 250 mL volumetric flask. The residue was washed via 3×5 mL of the same solvent and then completed to the final volume giving an initial working solution, containing 0.4 and 2 mg/mL of MT and PC, respectively.

(a) HPLC method.—The prepared working solution was 5-fold diluted using mobile phase and 20 μ L was injected into the instrument. The separation was achieved under the above-mentioned chromatographic conditions.

(b) TLC densitometry method.—Ten μ L from the prepared working solution were spotted on the TLC plate. The development and quantification were assessed under the for-mentioned chromatographic conditions.

For both methods, the standard addition technique was done. The assessment was carried out on pharmaceutical instead of preparing placebo, thus, known accurate quantities of each standard were spiked into pharmaceutical formulation. The recovery assessment was carried out on three different concentration levels of addition for each standard.

Results and Discussion

The current study purposes to develop and validate two chromatographic methods for the simultaneous determination of MT and PC in the presence of PC impurities.

Method Development and Optimization

After evaluation of several solvent compositions, 10% aqueous methanol at which all standards exhibited good solubility under the investigated concentrations, was chosen for preparing the stock and working standard solutions. The investigated compounds exhibit a significant difference in lipophilicity (log P) and ranging from -1.85, 0.51, 0.47, and 1.93 for MT, PC, AP, and NP, respectively (28).

In the HPLC-UV method, a “dual-mode” gradient was employed to improve the resolution and shorten the time of analysis (1), (29–34). Changing both mobile phase composition and flow rate creates more chance to obtain optimum separation in the least possible time and provides more solutions for the separation of overlapped peaks. Furthermore, the ternary mobile phase systems permitted better separation compared to their binary counterparts (35–39).

Trials for the HPLC method development were starting via using normal binary combinations of H₂O/MeOH or H₂O/ACN in different gradient programs but these trials suffered from either loss of resolution and/or long analysis time. The best chromatographic parameters were achieved via ternary combination of H₂O/ACN/MeOH but still suffering a long analysis time. Though changing the flow rate via the dual-mode gradient offers a relatively short analysis time alongside no potential increase in back pressure was noticed (maximum back pressure reached was 350 bar). The flow rate was increased after emerging the third analyte, from 1.5 to 2.5 mL/min. This flow rate represents the best compromise between reducing analysis time and a tolerable increase in column backpressure. Also, optimum flow rate after 2.7 min was found to be 1.5 mL/min where the optimum resolution between PC and AP peaks was obtained.

The optimum separation was achieved via a ternary mobile phase constituted of methanol–acetonitrile–water. The percentage of each component and the mobile phase flow rate were

modified with time as described in Table 1. Figure 1a and 1b show representative chromatograms realized with standards without and with the impurities, respectively.

In the TLC method, the optimum mobile phase composition was (butanol–dioxane–toluene–methanol; 8:2.5:3.5:0.3 by volume) revealing satisfactory chromatographic separation between PC and MT without and with PC impurities as presented in Figures 2a, 2b, 3 and 4. MT demonstrated a relatively high affinity towards the polar silica and showing a good separation from PC and its impurities.

Method Validation

Validation was conducted according to ICH guidelines (26) and the validation parameters were assessed for the two proposed methods as shown in Table 2.

(a) Linearity.—Linearity was realized in the range of 10–200 and 20–600 $\mu\text{g/mL}$ for MT and PC, respectively in the HPLC method and range of 2–12 $\mu\text{g/spot}$ for MT and 5–30 $\mu\text{g/spot}$ for PC in the TLC method. Linear regressions equations were obtained from the constructed calibration curves.

(b) Accuracy.—In the TLC method, accuracy was assessed via the mean percentage recovery and the standard deviation. The Mean \pm SD was found 100.69 ± 1.26 and 99.83 ± 1.64 for MT and PC, respectively. Whereas, in the HPLC method were 100.88 ± 1.07 for MT and 101.51 ± 0.38 for PC. Both methods were found to be accurate.

(c) Precision.—Upon examination of repeatability, RSD of MT was 1.27 in TLC method and was 0.22 in HPLC method and that of PC was 0.59 in TLC method and was 1.44 in HPLC method.

For inter-day precision testing, RSD was found to be 0.09 for MT and 1.16 for PC in the TLC method and 1.37, 0.98 for MT and PC, respectively, in the HPLC method. These data suggest that both methods are precise.

(d) Specificity.—Data obtained from the analysis of the laboratory prepared mixes indicate that both methods are specific, Table 2.

(e) LOD and LOQ.—LOD and LOQ were calculated for each method as mentioned before and the results were represented in Table 2.

(f) Robustness and system suitability testing.—The robustness was assessed by evaluating the impact of the application of small changes on the chromatographic conditions as percent of the mobile phase components (H_2O , $70 \pm 1\%$), flow rate of mobile phase (2 ± 0.1 mL/min), and detector wavelength (220 ± 4 nm) in the HPLC method. For the TLC method, a small variation in the percent of mobile phase components (butanol, $56 \pm 1\%$), and time (60 ± 5 min) required for saturation with mobile phase were performed. The two methods were found robust as the investigated parameters did not reveal considerable differences in results regarding RSD, Table 3.

System suitability parameters including tailing factor, selectivity factor, resolution of peaks, and the number of theoretical plates were computed for the HPLC method and successfully fulfilled FDA recommendations (40) as shown in Table 4.

Assay of Pharmaceuticals

Both methods were employed for the determination of MT and PC in Hepamol[®] tablet. Standard addition technique was used to assess the validity of the developed methods to determine MT and PC selectively in presence of formulation excipients and additives, where satisfactory results were found, Table 5.

Statistical Comparison

One-way ANOVA Statistical comparison at 95% confidence interval (41) was performed on the recovery percent data acquired from the application of the two described methods on pharmaceuticals as shown in Table 6. The comparison showed that there was no significant difference between results obtained from the developed methods and the manufacturer's

method. The proposed methods can be used accurately for the assessment of MT and PC in their binary mixes and in pharmaceutical preparations.

Conclusion

Two robust, precise and selective impurity indicating chromatographic methods were developed and validated for the determination of MT and PC in its pharmaceutical preparation without interference from PC impurities. The methods were validated as per the ICH guidelines. Results reveal that the dual-mode gradient in combination with a ternary mobile phase enabling good resolution of different lipophilic compounds within a relatively short analysis time. The TLC method displayed good chromatographic parameters and validated as per the ICH. However, the HPLC method was found to be advantageous concerning the analysis time than the TLC method. Both methods were found appropriate to be employed as impurity indicating methods for determination of paracetamol in pure form or in pharmaceutical dosage forms with methionine in quality control laboratories.

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Figure captions

Figure 1. HPLC chromatograms of **A)** MT (90 µg /mL) and PC (90 µg /mL); **B)** MT (100 µg /mL), PC (100 µg /mL), AP (100 µg /mL) and NP (100 µg /mL) using specified chromatographic conditions.

Figure 2. Two dimensions TLC chromatograms showing **A)** separation of MT (6 µg / spot, R_F : 0.26) and PC (20 µg / spot, R_F : 0.82); **B)** separation of MT (4 µg / spot, R_F : 0.26) and PC (15 µg / spot, R_F : 0.83) from the two paracetamol impurities (AP, R_F : 0.59 & NP, R_F : 0.93).

Figure 3. Three dimensions TLC chromatogram showing the separation of MT (different µg / spot, R_F : 0.26) and PC (different µg / spot, R_F : 0.83) from the two paracetamol impurities (AP, R_F : 0.59 & NP, R_F : 0.93).

Figure 4. Three dimensions TLC chromatogram showing the separation of MT in range (2-12 µg / spot) at R_F : 0.26 and PC in range (5-30 µg / spot) at R_F : 0.83.

Table 1. Dual-mode gradient program of the HPLC method.

Time (min)	Acetonitrile (%)	Methanol (%)	H ₂ O (%)	Flow rate (mL/min)
0 – 2.7	15	15	70	2
2.7 – 3	15	10	75	1.5
3 – 4.6	15	10	75	1.5
4.6 – 5.6	25	25	50	2.5
5.6 – 10	25	25	50	2.5

Table 2. Assay validation of the proposed methods for the determination of MT and PC as per ICH guidelines.

Parameter	TLC		HPLC	
	MT	PC	MT	PC
Accuracy ^a	101.28 ± 0.59	100.62 ± 0.14	99.96 ± 0.86	100.44 ± 1.26
Precision				
Repeatability ^b (%RSD)	1.27	0.59	0.22	1.44
Intermediate Precision ^c (%RSD)	0.09	1.16	1.37	0.98
Linearity				
Correlation Coefficient	0.9991	0.9999	0.9997	0.9999
Slope	1205.0625	1158.2805	2.6228	2.5843
Intercept	5891.1464	5926.2643	9.6375	13.3120
Range	2 - 12 µg / spot	5 - 30 µg / spot	10-200 µg /mL	20- 600 µg /mL
LOD	0.6125 µg / spot	1.5248 µg / spot	2.1711 µg /mL	2.1822 µg /mL
LOQ	1.8560 µg / spot	4.6206 µg / spot	6.5791 µg /mL	6.6128 µg /mL
Specificity ^d	101.25 ± 1.66	100.93 ± 0.58	100.87 ± 1.08	100.52 ± 1.92

^a Accuracy (mean of percent recoveries ± SD) assessed using a minimum of nine determinations over a minimum of three concentration levels covering the specified range.

^b The intraday (n = 3), RSD on three different concentration levels within the specified range for each standard repeated three times within the same day.

^c The interday (n = 3), RSD on three different concentration levels within the specified range for each standard repeated three times in three consecutive days.

^d Specificity (mean of percent recoveries ± SD) of recovery percent data of the laboratory prepared mixes.

Table 3. Parameters associated with robustness assessment.

Method	TLC						HPLC									
Variable parameter	Mobile phase saturation time (min)			Mobile phase composition (% , butanol)			Mobile phase flow rate (mL / min)			Mobile phase composition (% , H ₂ O)			Detector wavelength (nm)			
Degree of variation	55	60	65	55	56	57	1.9	2	2.1	69	70	71	216	220	224	
Measured value	R _F value						t _R (min)									
	MT	0.26	0.27	0.26	0.25	0.27	0.26	1.72	1.70	1.65	1.73	1.70	1.72	1.68	1.70	1.71
	PC	0.82	0.83	0.86	0.82	0.83	0.82	2.65	2.63	2.59	2.64	2.59	2.58	2.57	2.59	2.61
Mean	MT	0.26			0.26			1.69			1.72			1.70		
	PC	0.84			0.82			2.62			2.60			2.59		
Standard deviation	MT	0.01			0.01			0.04			0.02			0.02		
	PC	0.02			0.01			0.03			0.03			0.02		
RSD (%)	MT	2.19			3.85			2.13			0.89			0.90		
	PC	2.49			0.70			1.16			1.23			0.77		

Table 4. System suitability parameters for the analysis of MT and PC using the proposed HPLC method.

Parameter	MT	PC	MT-PC	PC-AP	AP-NP	Recommended value ^a
Tailing factor (T)	1.05	1.16				≤ 2
Theoretical plates number (N)	2900	3050				> 2000
Resolution (R_s)			3.672	2.144	6.840	> 2
Selectivity factor (α)			2.66	1.66	2.54	> 1

^a Recommended values defined by FDA Center of Drug Evaluation and Research's reviewer guidance on validation of chromatographic methods (November 1994) (40).

Table 5. Determination of MT and PC in pharmaceuticals by the proposed methods and application of standard addition technique.

Drug/Method	TLC (Recovery % \pm SD)	HPLC (Recovery % \pm SD)
MT (Hepamol [®] tablet)	100.76 \pm 1.19	101.02 \pm 0.72
PC (Hepamol [®] tablet)	102.03 \pm 1.82	100.94 \pm 1.47
MT (Standard addition technique) ^a	100.69 \pm 1.26	100.88 \pm 1.07
PC (Standard addition technique) ^a	99.83 \pm 1.64	101.51 \pm 0.38

^a (mean \pm SD) estimated using nine determinations over three different concentration levels covering the specified range.

Table 6. One-way ANOVA statistical analysis within 95% confidence interval on recovery percent data obtained from the manufacturer's method and application of the two corresponding methods on pharmaceutical preparation.

One Way ANOVA						
Dependent variable: Recovery percent data						
	Source of variation	Sum of Squares	df ^a	Mean Square	F ^b	P-value
MT	Between groups	0.050	2	0.025	0.082 (5.14 ^c)	0.923
	Within groups	1.850	6	0.308		
	Total	1.901	8			
PC	Between groups	4.060	2	2.030	1.898 (5.14 ^c)	0.230
	Within groups	6.418	6	1.070		
	Total	10.478	8			

^a Degrees of freedom.

^b F calculated is the ratio of mean square to error mean square.

^c The tabulated value of F.

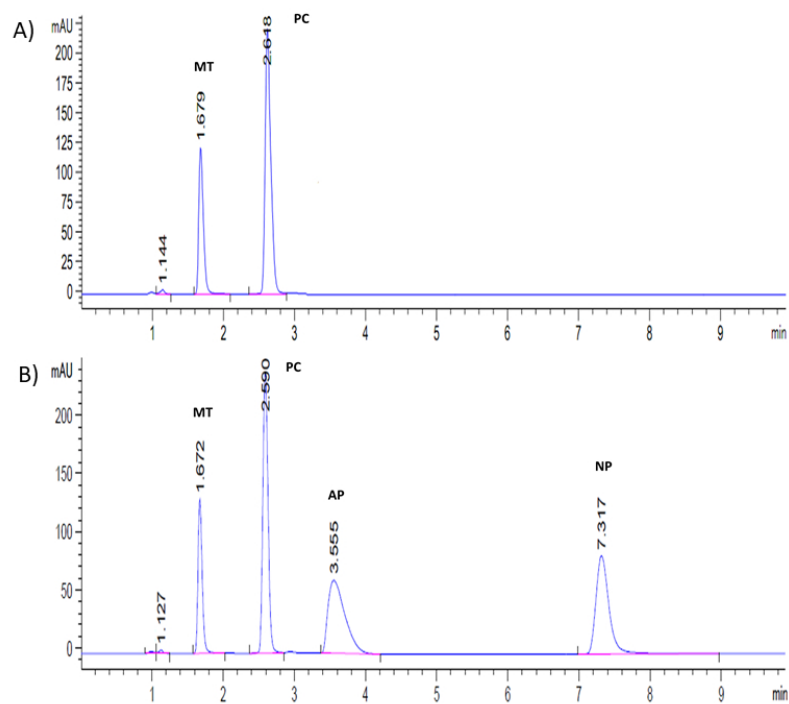


Figure 1. HPLC chromatograms of A) MT (90 $\mu\text{g}/\text{mL}$) and PC (90 $\mu\text{g}/\text{mL}$); B) MT (100 $\mu\text{g}/\text{mL}$), PC (100 $\mu\text{g}/\text{mL}$), AP (100 $\mu\text{g}/\text{mL}$) and NP (100 $\mu\text{g}/\text{mL}$) using specified chromatographic conditions.

254x190mm (96 x 96 DPI)

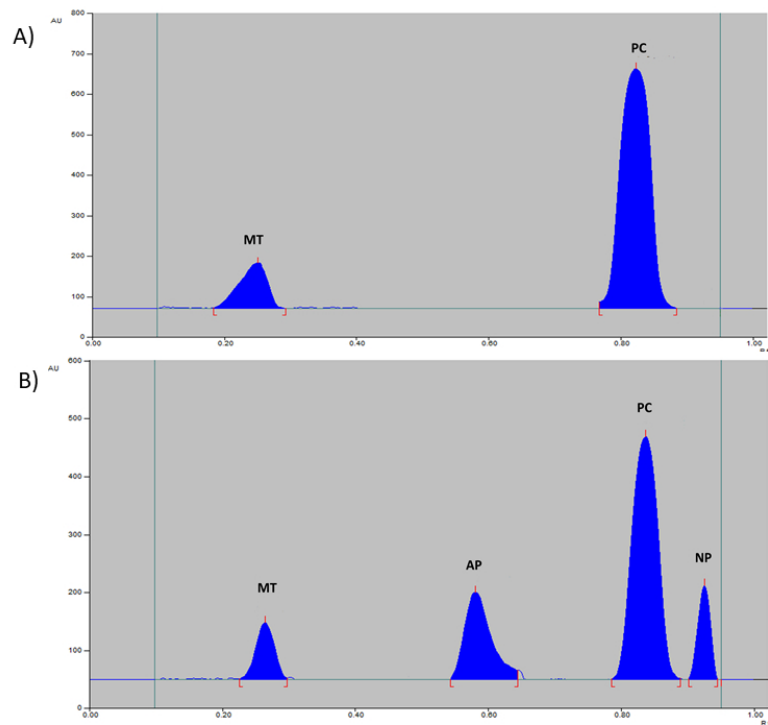


Figure 2. Two dimensions TLC chromatograms showing A) separation of MT (6 µg/spot, R_f: 0.26) and PC (20 µg/spot, R_f: 0.82); B) separation of MT (4 µg/spot, R_f: 0.26) and PC (15 µg/spot, R_f: 0.83) from the two paracetamol impurities (AP, R_f: 0.59 & NP, R_f: 0.93).

254x190mm (96 x 96 DPI)

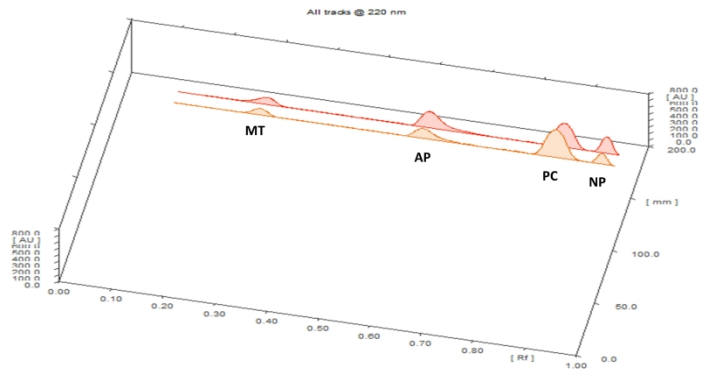


Figure 3. Three dimensions TLC chromatogram showing the separation of MT (different $\mu\text{g}/\text{spot}$, RF: 0.26) and PC (different $\mu\text{g}/\text{spot}$, RF: 0.83) from the two paracetamol impurities (AP, RF: 0.59 & NP, RF 0.93).

338x190mm (300 x 300 DPI)

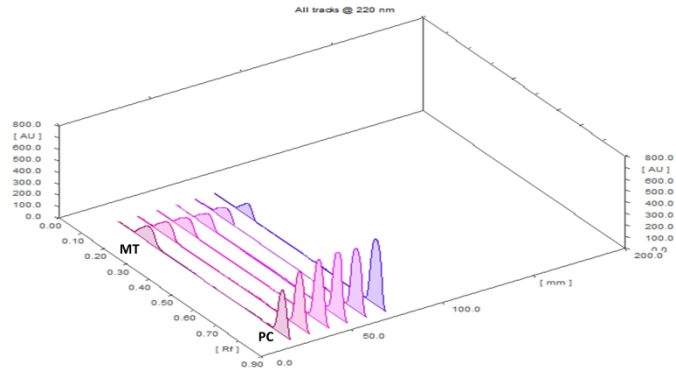


Figure 4. Three dimensions TLC chromatogram showing the separation of MT in range (2-12 $\mu\text{g}/\text{spot}$) at RF: 0.26 and PC in range (5-30 $\mu\text{g}/\text{spot}$) at RF: 0.83.

338x190mm (300 x 300 DPI)