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EVALUATED METHOD FOR THE SEPARATION AND IDENTIFICATION OF PARACETAMOL IMPURITIES BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (RP-HPLC)

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ABSTRACT

Paracetamol is used as pain and fever treatment and listed as the most important medication needed in the basic health system. This study was established to separate paracetamol and some impurities from a mixture using high performance liquid chromatography (HPLC) via Ultra Violet (UV) detection. A gradient reverse-phase high performance liquid chromatography (RP-HPLC) with UV detector performed to detect paracetamol and impurities. The eluents consisted of water/methanol/ammonium acetate 95:5: 5m v/v/mM (eluent A) and Methanol/water/ ammonium acetate 95:5: 5 v/v/mM (eluent B) usinga gradient program at a flow rate of 300 µL/min with 238 nm detection and an injection volume of 20 µL. A fast and repeatable method for the separation by HPLC using reverse-phase (RP) was developed first. The impurities had been successfully separated.

Keywords: Gradient; Impurities; Paracetamol; RP-HPLC

INTRODUCTION

Paracetamol depicted in Figure 1 is widely used as analgesic and antipyretic drug **[1-2]** and it is listed as the most important medications needed in the basic health system in the World Health Organization

(WHO) model list of essential medicines **[3-8]**.

As paracetamol could be hepatotoxic **[9]**, the concentrations of the related constituents, 4-aminophenol, 4-nitrophenol and 4-chloracetanilid should be lower than 0.005%, 0.05% and 0.001%, respectively, particularly the 4-aminophenol which is paracetamol main degradation product **[10]** that might be toxic for kidney also **[11]**. A purity testing method is essential for registration and is important for drug product development.

The presence of acetaminophen impurities primarily the 4-aminophenol (4-AP) arises in pharmaceutical dosage formsas a result of both synthesis, degradation during storage **[12, 13]**, quality of the raw materials, the solvents and reagents used, the equipments, the reaction conditions and the root of synthesis **[14]**. Synthesis of paracetamol can be done by many methods; acetylation of p-aminophenol which produced from different raw materials such as phenol, chlorophenol, chlorobenzene or aniline **[4, 8, 15, 16, 17, 18],** also the direct acylation of phenol with acetic anhydride catalysed by hydrofluoric acid **[5]**. These methods may be a source of impurities.

A chromatographic technique for measuring the concentrations of acetaminophen impurities (4-nitrophenol (4-NP), 4'-chloroacetanilide (4-CA) and 4-aminophenol) described in British Pharmacopeia requires 30 minutes for analysis **[19]**. In addition, numerous methods applied to detect impurities of acetaminophen in pharmaceutical dosage using high performance liquid chromatography with different conditions **[14, 20, 21, 22]**. Numerous methods using different techniques to test the impurities including: electrophoresis **[23, 24]**, flow injection analysis with spectrophotometric detection **[25]**, fluorimetric analysis **[26]**, Ultraviolet spectrometry **[27, 28]**, and micellar electro-kinetic chromatography **[12]**.

Although all the mentioned active pharmaceutical ingredients have been available on the market for decades, new methods for determination of their impurities in dosage forms, following the current legislation, still need to be developed to ensure the safety of drug products. The aim of this study was to develop and validate an analytical method for quantitative purity testing of paracetamol.

MATERIALS AND METHODS

Instruments

High Performance Liquid Chromatography (HPLC), HP 1100 HPLC-UV. Eluent A: Water/Methanol (95:5; V:V) + 5 mM ammonium acetate. Eluent B:

Water/Methanol (5:95; V: V) + 5 mM ammonium acetate.

Column: MZ Perfect Aqua C18, 50 x 2.1 mm, 5 µm particle size + precolumn 10 x 2.1 mm

Methods

Table1 illustrates the HPLC parameters of the final method which was developed (chromatographic conditions)

Chemicals

'Paracetamol mix' (paracetamol with impurities) (Hikma Pharmaceutics Group-Jordan), Paracetamol reference, 98 %were purchased from ICNP (Milano, Italy), Methanol (MeOH) and Ultrapure water. All reagents used were of analytical-reagent grade and were used as obtained. Acetonitrile solvent and methanol were of HPLC grade. Water was de-ionized and doubly distilled from glass apparatus. All solvents and solutions for HPLC analysis were filtered through a Millipore filter (pore size 0.40 gm) and vacuum-degassed by ultrasound treatment.

Experimental Procedure

The stock solutions of 'paracetamol mix' (mixture) and the paracetamol reference with a concentration of respectively 2.5 mg/mL were prepared using MeOH. The 'paracetamol mix' was diluted in H_2O/MeOH (1:1, V: V) to a concentration of 10 µg/mL (mixture 1). Additionally, a solution of 'paracetamol mix' in H_2O/MeOH (95:5, V:V) was prepared. For the multiple measurements and the collection of the fractions, the concentration was increased to 20 µL/mL (mixture 2). The reference was prepared with a concentration of 5 µL/mL in H_2O/MeOH (95:5, V:V).

The starting HPLC conditions at the beginning of the method development are shown in Table 2. Apart from the sampling rate, the gradient of the mobile phase and the parameters flow rate and injection volume were changed for the new method.

During the method development, the gradient was changed to get a baseline separation of all impurities in the mixture. Gradient 1 should give an idea of the behaviour of the substances contained in the mixture. In the next step, the equilibration time was adjusted. Therefore, the washing time after receiving a concentration of 100 % eluent A at 12 minutes was held constantly for four minutes. These settings are expressed in gradient 2. For gradient 3, the starting concentration of eluent A was decreased to 70 %. In addition, the slope of this gradient was decreased from 10 % eluent A/min to 7 % eluent A/min. Gradient 4 held the starting concentration of eluent A for 2 minutes at 100 %. Afterwards, the concentration was lowered to 20 % with a slope of 10 % eluent A/min. Both gradients

had a plateau at the lowest aqueous concentration for one minute before the equilibration time of four minutes was performed. Gradient 5 is comparable to gradient 4. The difference is the end concentration, which was set to 40 % eluent A. Additionally, the plateau at the lowest concentration was removed. All gradients are represented graphically in Figure 5. The detailed gradients are added to the in Table 3.

RESULTS AND DISCUSSION

HPLC method development

Equilibration the HPLC was established before starting measurements. Afterwards, a blank of H_2O/MeOH (1:1, V:V) using the starting HPLC conditions (Table 2) with gradient 1 was recorded to get an underground chromatogram of the solvent. The same gradient was applied for mixture 1. As can be seen in Figure 2, the substances eluted at a low retention time visible by comparison of the chromatograms of mixture 1 and the blank (Figure 3). The significantly higher intensity of the peak at 1.09 min shows that the substances coelute with MeOH and in a broad peak at 3.05 min.

The starting phase of the gradient is composed of a high percentage of eluent A, which has a lower eluting power than MeOH on a reversed-phase column. Therefore, it is unusual that the substances coelute with MeOH near the dead time, since paracetamol should interact with the C18 column due to van der Waals forces. The hypothesis was made that the actual composition of the mobile phase does not match with the settings of the gradient. It can result from a missing equilibration time after changing the solvent composition from organic to aqueous. The instrument has a greater delay than expected because of the death volume time. Thus, an equilibration time of four minutes was added to gradient 1. The duration of the equilibration time can be proved by a constant pressure, since water and MeOH have different pressure in the column due to different viscosity. Therefore, mixture 1 was measured with gradient 2 considering the equilibration time. In Figure 4, the HPLC-UV chromatogram of mixture 1 is illustrated. It becomes apparent that mixture 1 contains numerous different impurities besides paracetamol. The assumed peaks are labelled by numbers 1–7.

However, the real number of substances in mixture 1 is unknown. To confirm that no additional substances elute at higher retention time, another measurement with a higher organic amount was performed to reduce the retention time of the substances and to find additional substances with stronger nonpolar properties (Figure 5).

Later eluting substances were not detected in this measurement. The peak with the highest retention time corresponds to peak 7 in Figure 4, which is confirmed by the comparison of the UV-spectra of these peaks. Due to the higher elution power of MeOH, the retention time is shifted to lower values. In comparison to Figure 8, the peaks 3, 4 and 7 in Figure 7 are baseline separated. Only peaks 5 and 6 are slightly overlapping. The broad peaks 1 and 2 have a low intensity and must be verified as substances during the development. Peaks 1–4 are broad and not symmetric. The reason for this can be the significant difference between the solvent of the sample and the composition of the mobile phase. Therefore, a new sample with a solvent of H_2O/MeOH (95:5, V:V) was prepared and measured under same conditions (Figure 6).

The peak shape of the peaks 1–4 in Figure 9 is improved. Furthermore, it becomes clear that the assumed peak 2 was underground noise and is not found anymore. Thus, mixture 1 contains six detectable substances. It is still the case that the peaks 5 and 6 need to be baseline separated. In order to separate the overlapping peaks, the composition of the mobile phase was kept constant at 100 % eluent A for two minutes. It was expected that thereby the differences in the polarity of the coeluting substances would be more distinct. This was performed in gradient 4, whereby the slope from gradient 1 was transferred. However, the end concentration of the mobile phase was set to 20 % eluent A, since all substances elute before reaching this concentration. Figure 7 displays the obtained chromatogram, which shows baseline separation of all six peaks. This means that the resolution has to be higher than 1.5. This is verified by the calculation for the resolution of the two closest peaks 4 and 5.

The next step was to shorten the run time of the method, while obtaining baseline separation of all peaks. This should be received by increasing the flow rate from 200 µL/min to 300 µL/min. Another advantage could be an improvement of the peak shape. The retention time decreased e.g. peak 6 from 11.27 min to 9.51 min by maintaining the baseline separation. There was no significant variation in the peak shape (Figure 8). Owing to the shorter retention time, the gradient could be adjusted to an end concentration of the mobile phase of 40 % eluent A. This had the aim of further lessen the analysis time. Figure 9 shows the chromatogram of mixture 1 with the new gradient 5 and the flow rate of 300 µL/min.

Since the development of the separation was completed, the injection volume as

another HPLC parameter was changed. It was increased to 20 µL, in order to enhance the signal/noise ratio. For the determination of the underground noise, a blank was measured (Figure 10). Afterwards, mixture 1 was analysed. It can be seen, that the intensity of the peaks grew and a superior signal/noise ratio was received (Figure 11). In addition to that, no overload with substance was detected.

Before the ruggedness of the developed method was examined by multiple measurements, the concentration of the mixture was doubled to get enough amount of substance in the fractions for the following analysis via HR-MS. A possible overload was excluded with a pre measurement of the new sample concentration, which is shown in Figure 12. Figure 12 is representative illustrated for the multiple measurement of mixture 2. The developed method is fast and rugged. This is confirmed by the value of the standard deviation of the retention times shown in Table 4.

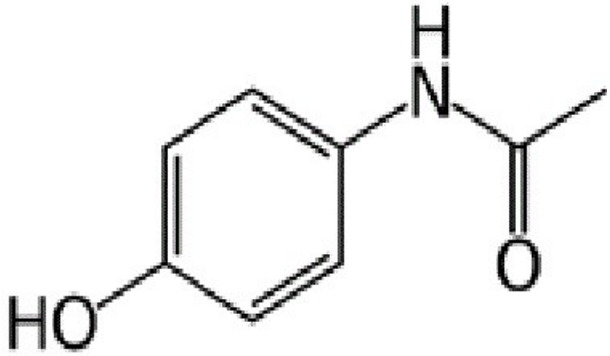

Figure 1 Structure of paracetamol (4-Acetamidophenol; N-(4-hydroxyphenyl)acetamide)

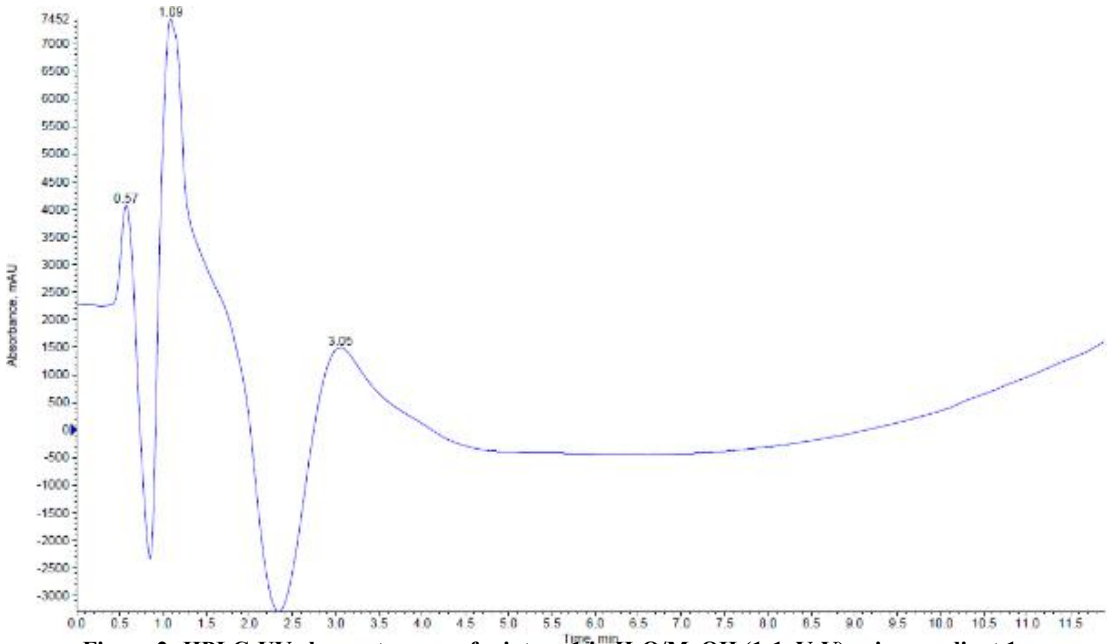

Figure 2: HPLC-UV chromatogram of mixture 1 in H_2O/MeOH (1:1, V:V) using gradient 1

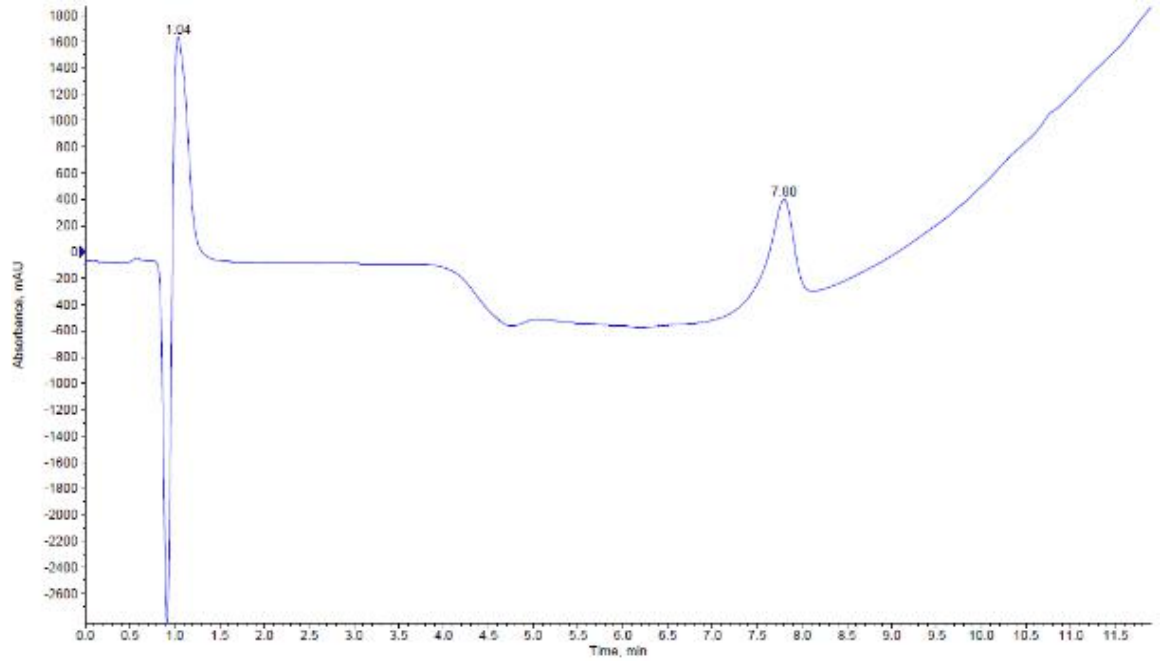

Figure 3: HPLC-UV chromatogram of blank H_2O/MeOH (1:1, V:V) using starting HPLC conditions and gradient 1

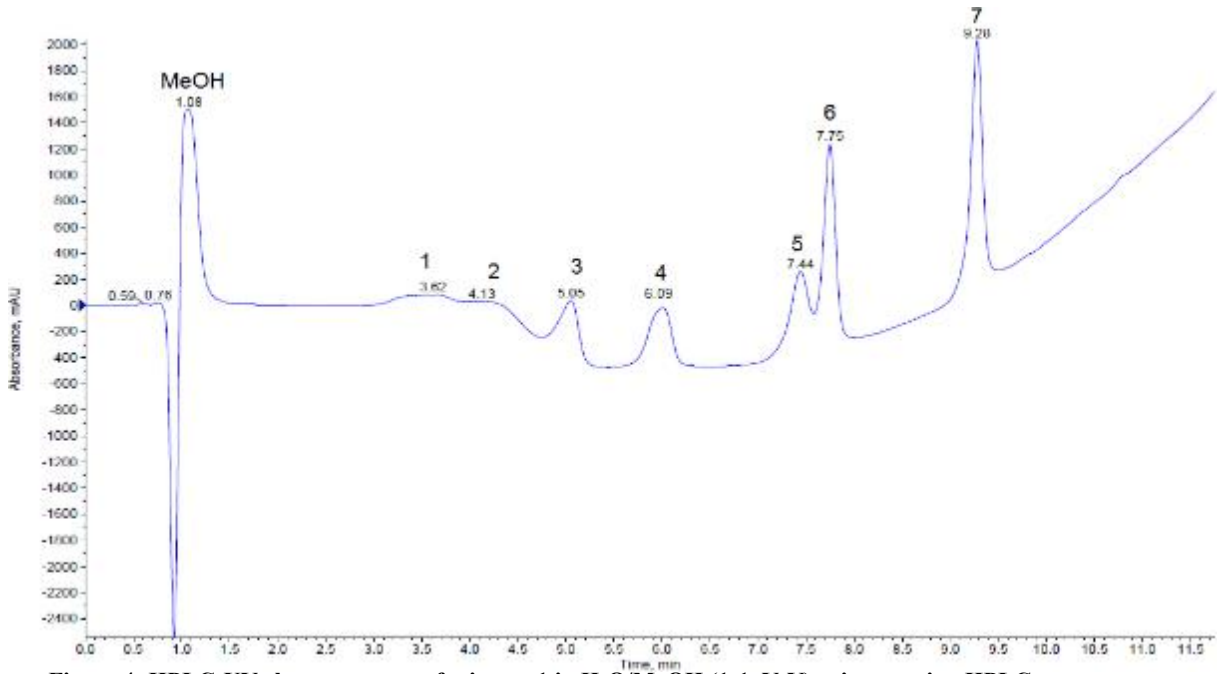

Figure 4: HPLC-UV chromatogram of mixture 1 in H_2O/MeOH (1:1, V:V) using starting HPLC conditions and gradient 2

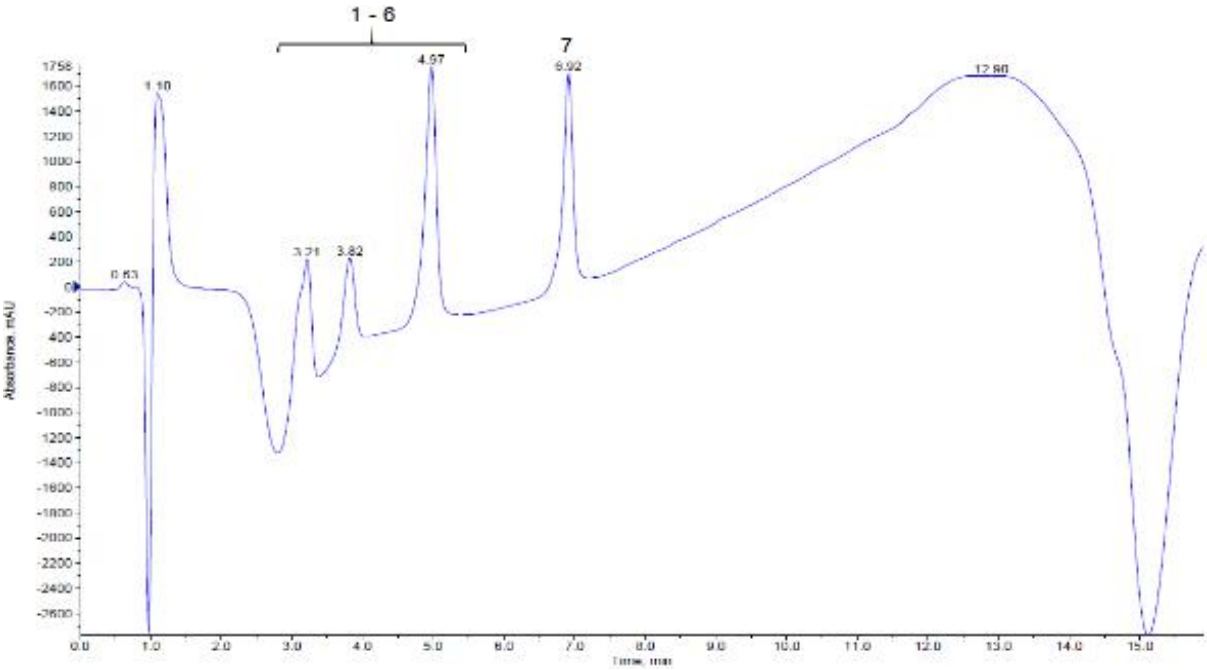

Figure 5: HPLC-UV chromatogram of mixture 1 in H_2O/MeOH (1:1, V:V) using starting HPLC conditions and gradient 3

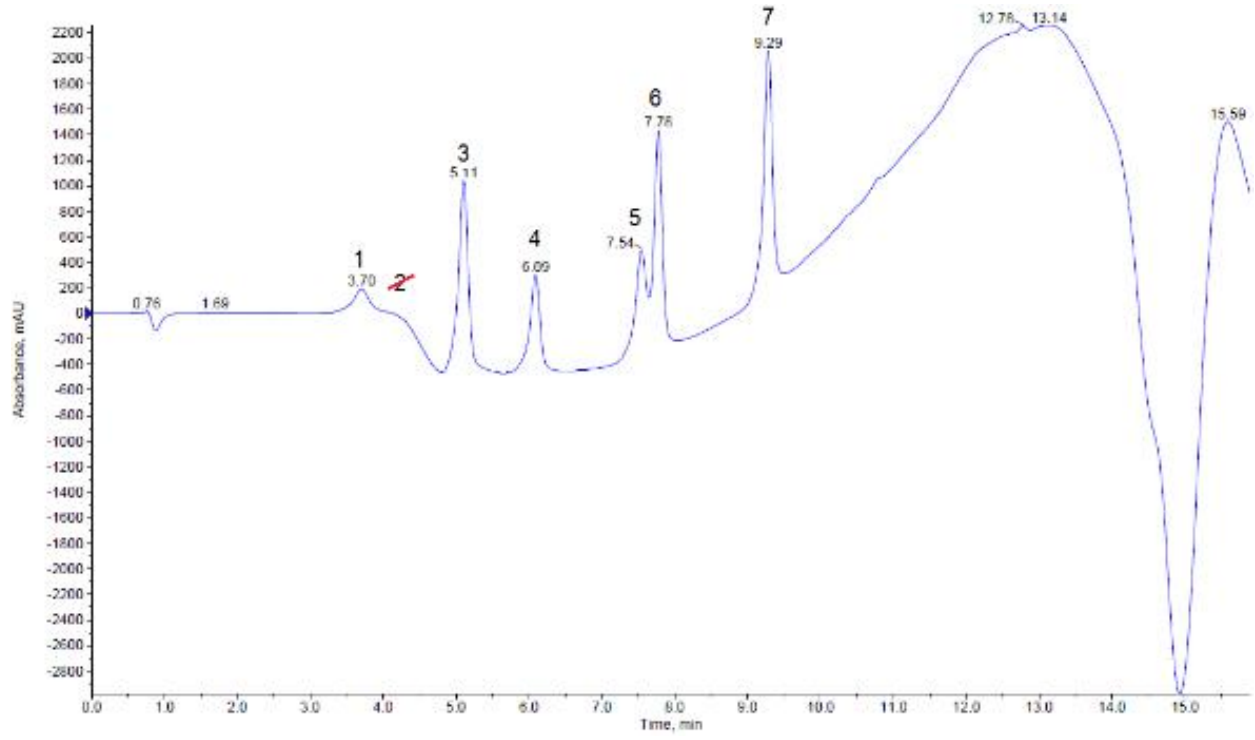

Figure 6: HPLC-UV chromatogram of mixture 1 in H_2O/MeOH (95:5, V:V) using starting HPLC conditions and gradient 2

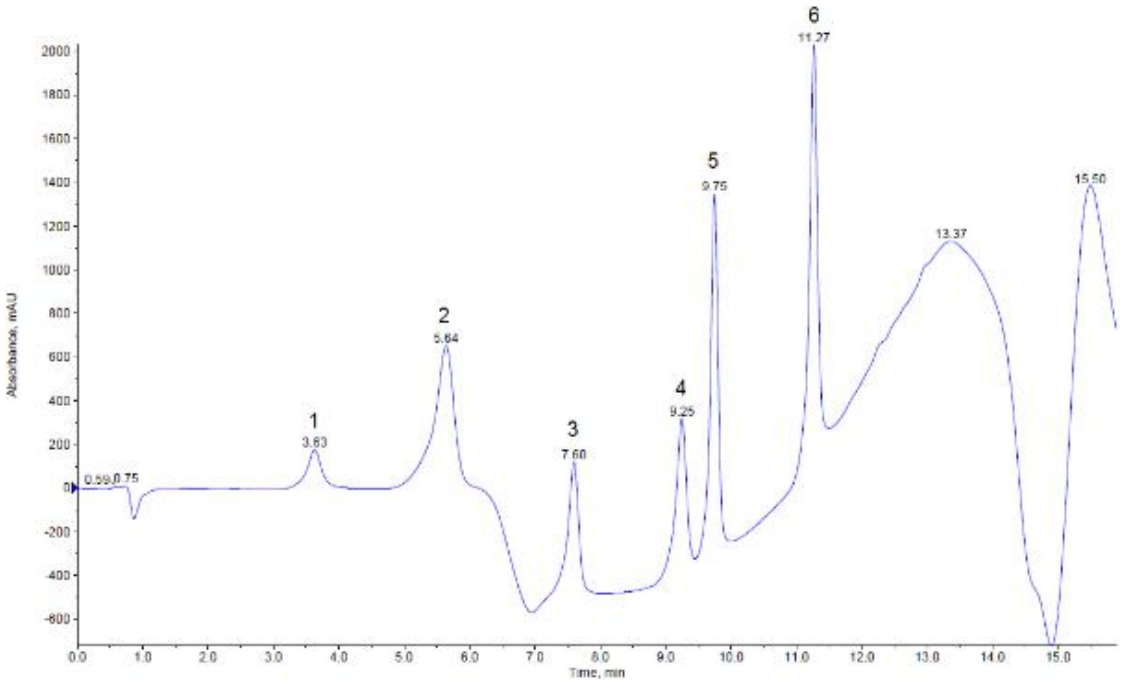

Figure 7: HPLC-UV chromatogram of mixture 1 in H_2O/MeOH (95:5, V:V) using starting HPLC conditions and gradient 4

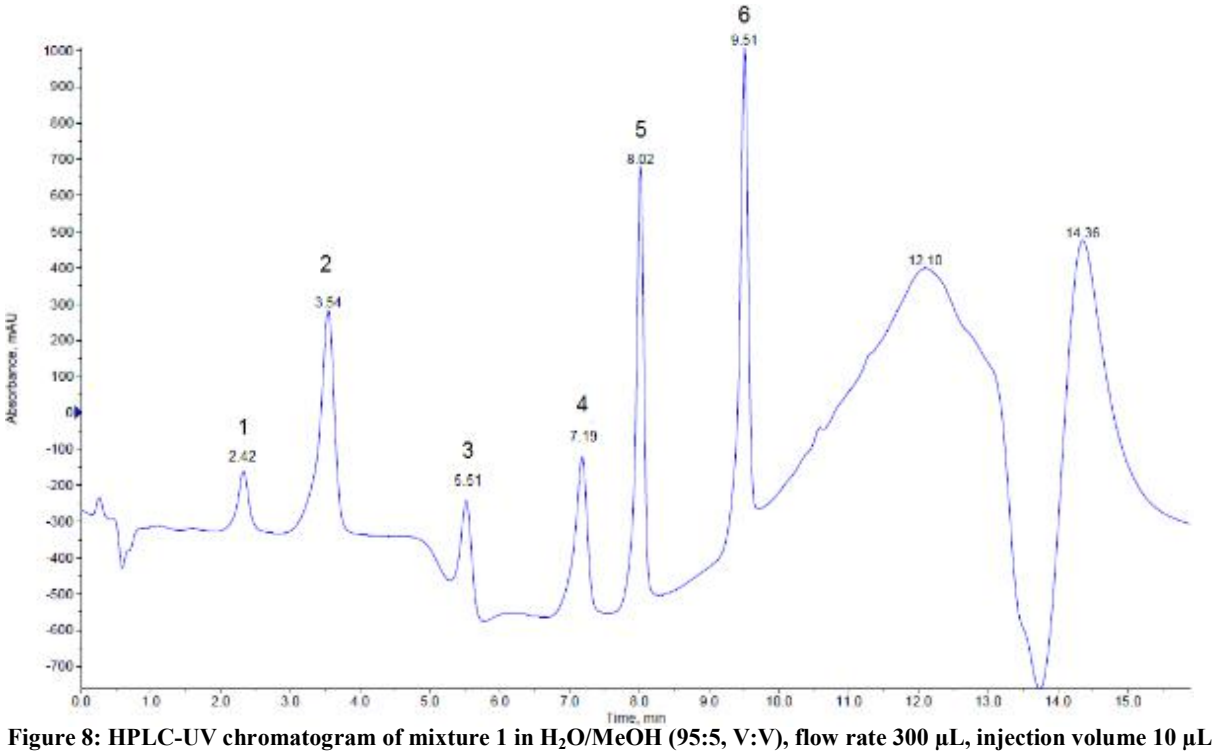

Figure 8: HPLC-UV chromatogram of mixture 1 in H_2O/MeOH (95:5, V:V), flow rate 300 µL, injection volume 10 µL and gradient 4

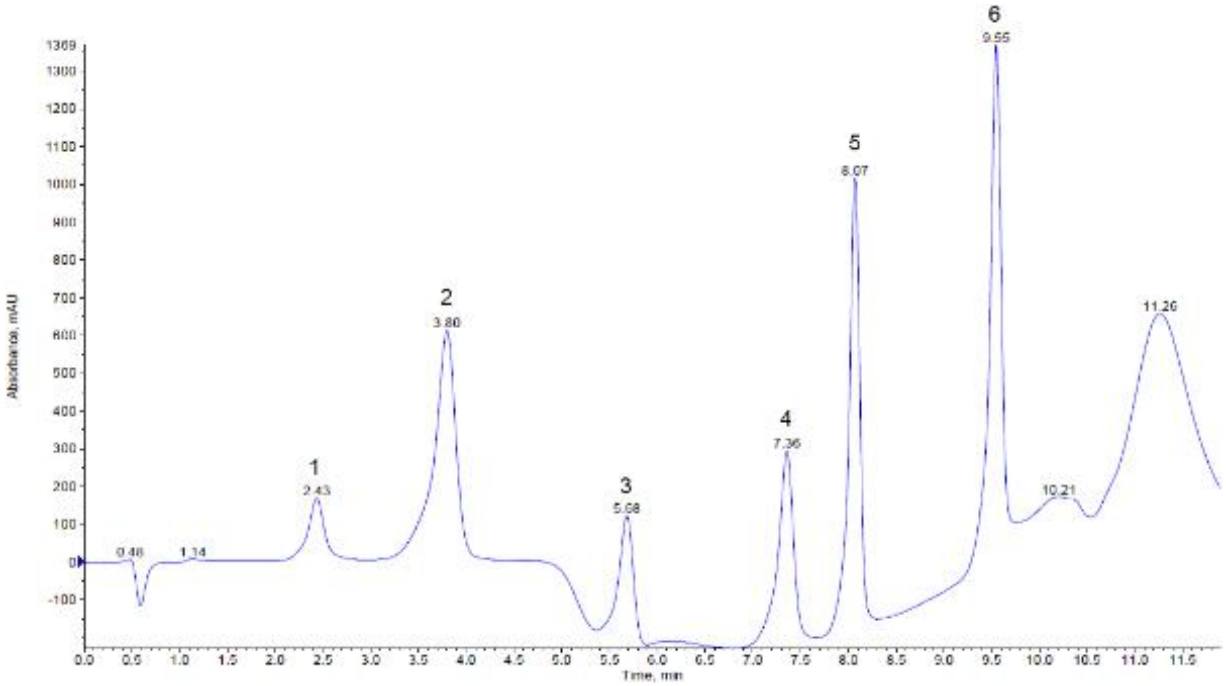

Figure 9: HPLC-UV chromatogram of mixture 1 in H_2O/MeOH (95:5, V:V), flow rate 300 µL, injection volume 10 µL and gradient 5

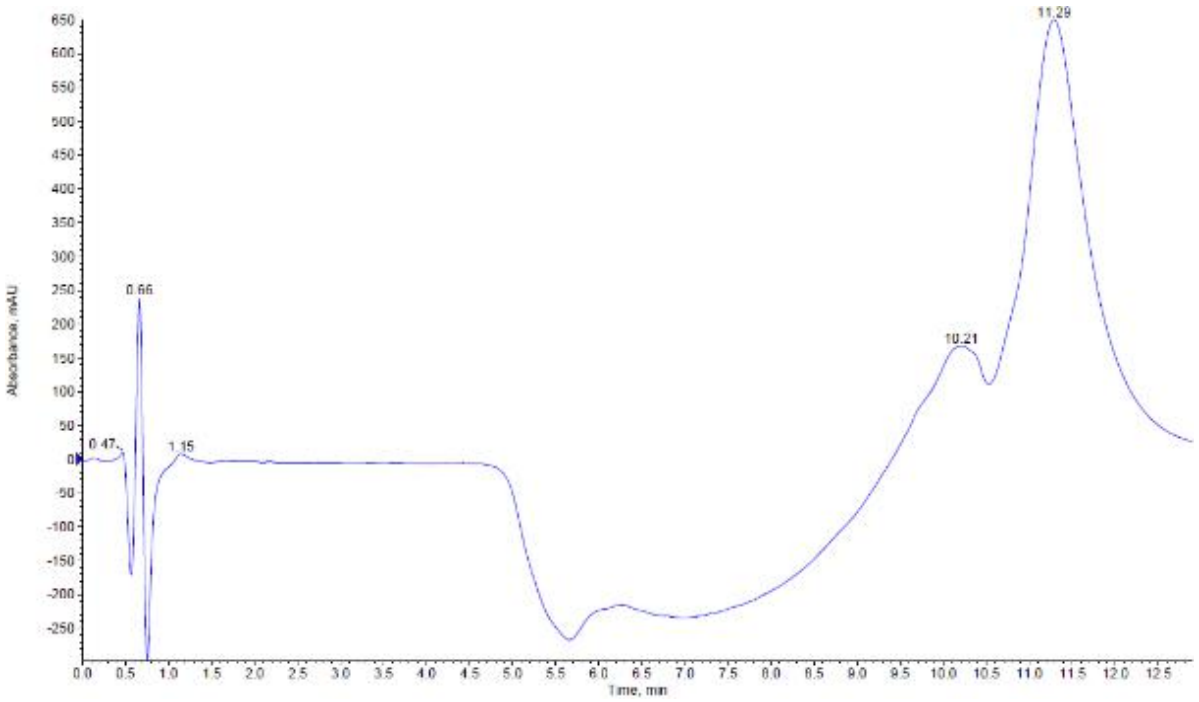

Figure 10: HPLC-UV chromatogram of blank H_2O/MeOH (95:5, V:V), flow rate 300 µL, injection volume 20 µL and gradient 5

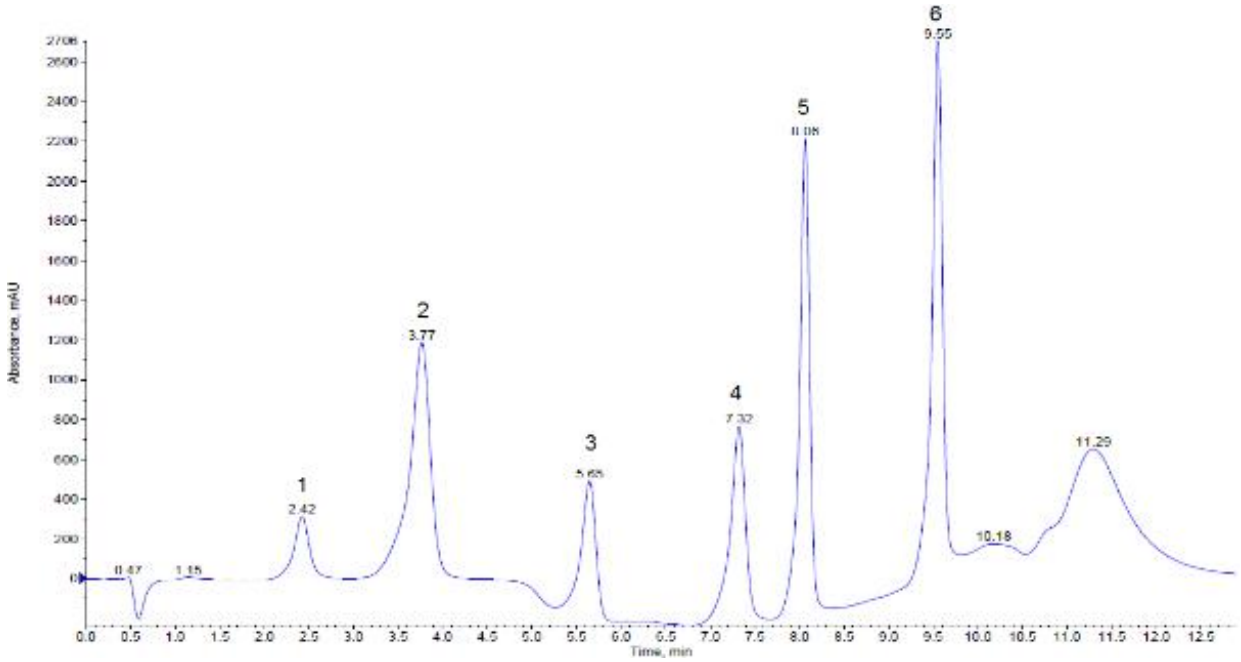

Figure 11: HPLC-UV chromatogram of mixture 1 in H_2O/MeOH (95:5, V:V), flow rate 300 µL, injection volume 20 µL and gradient 5

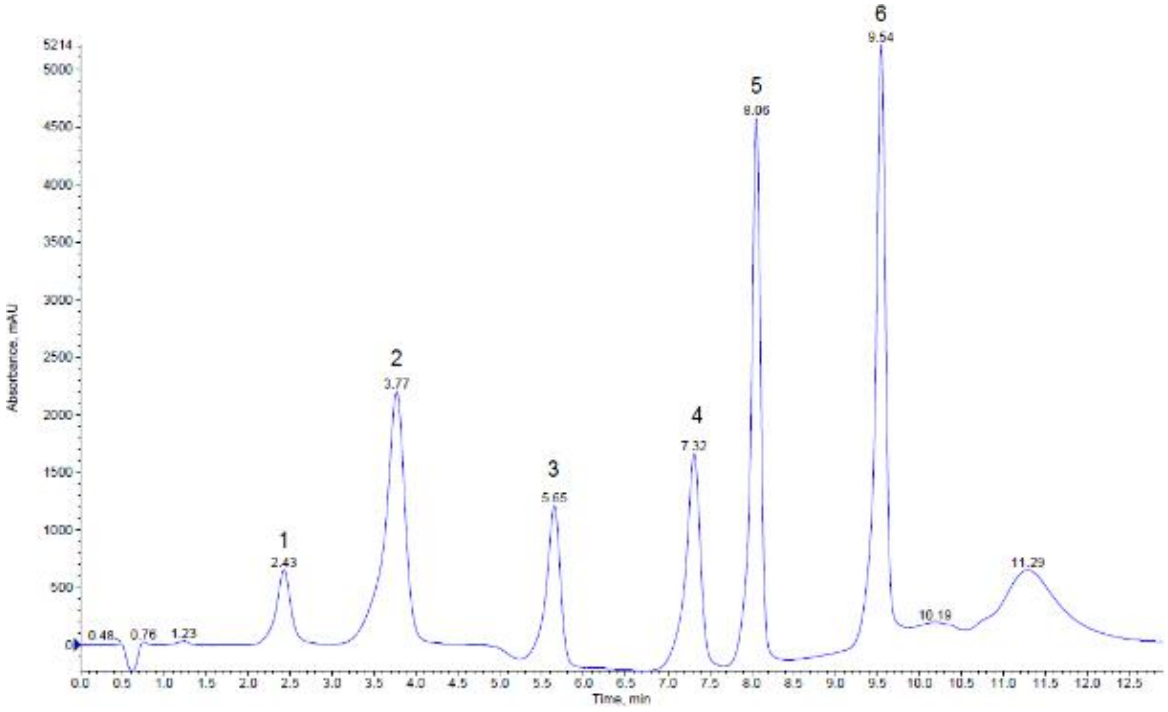

Figure 12: HPLC-UV chromatogram of mixture 2 in H_2O/MeOH (95:5, V:V), flow rate 300 µL, injection volume 20 µL and gradient 5, measurement A

Table 1: HPLC parameters of final method(chromatographic conditions)

Sampling rate	**1.25 Hz**		
Flow rate	**300 µL/min**		
Injection volume	**20 µL**		
Gradient	**Time (min.)**	**Eluent A (%)**	**Eluent B (%)**
	0.00	**100**	**0**
	2.00	**100**	**0**
	8.00	**40**	**60**
	9.00	**100**	**0**
	13.0	**100**	**0**

Table 2: Starting HPLC conditions

Sampling size	**1.25Hz**
Flow rate	**200µL/min.**
Injection volume	**10µL**

Table 3: Illustration of the used gradients

Gradient(I)	***Time (min.)***	***Eluent A (%)***	***Eluent B (%)***
	0.00	**100**	**0**
	10.0	**00**	**100**
	11.0	**00**	**100**
	12.0	**100**	**0**
	16.0	**00**	**100**
Gradient(II)	***Time (min.)***	***Eluent A (%)***	***Eluent B (%)***
	0.00	**100**	**0**
	10.0	**00**	**0**
	11.0	**00**	**100**
	12.0	**100**	**0**
	16.0	**100**	**0**
Gradient(III)	***Time (min.)***	***Eluent A (%)***	***Eluent B (%)***
	0.00	**70**	**30**
	10.0	**00**	**100**
	11.0	**00**	**100**
	12.0	**70**	**30**
	16.0	**70**	**30**
Gradient(IV)	***Time (min.)***	***Eluent A (%)***	***Eluent B (%)***
	0.00	**100**	**0**
	10.0	**20**	**80**
	11.0	**20**	**80**
	12.0	**100**	**0**
	16.0	**100**	**0**

Table 4: Comparison of retention times [min] of each peak during the quadruple measurement A-D

Peak / **Measurement**	**1**	**2**	**3**	**4**	**5**	**6**
A	**2.43**	**3.77**	**5.65**	**7.32**	**8.06**	**9.54**
B	**2.44**	**3.79**	**5.68**	**7.35**	**8.08**	**9.56**
C	**2.39**	**3.70**	**5.56**	**7.27**	**8.03**	**9.52**
D	**2.38**	**3.68**	**5.55**	**7.25**	**8.01**	**9.51**
Mean	**2.41**	**3.74**	**5.61**	**7.30**	**8.05**	**9.53**
Standard deviation	**0.03**	**0.05**	**0.06**	**0.05**	**0.03**	**0.02**

CONCLUSION

The aim of the experiment was the RP-HPLC separation of a paracetamol mixture including different impurities and the structure elucidation of these unknowns via HR-MSn. For this purpose, a RP-HPLC method was developed. The gradient was changed to receive a baseline separation of the substances in the paracetamol mixture. Best results were obtained by gradient 5 starting at 100 % eluent A and a decrease of 10 % eluent A/min. The decrement was already stopped at 40 %, since previously no additional nonpolar substances were detected by UV. This results in a faster method. The equilibration time for the column was included in the gradient for multiple measurements. Additionally, the parameters injection volume and flow rate were adjusted. By means of a triple measurement, the robustness of the developed method was confirmed. At the same time, the six found substances were collected in fractions for the structure elucidation. The structures of the examined substances were verified with the elution order of the previously performed RP-HPLC (Data not Published). In addition to that, the synthesis pathway of paracetamol and the resulting impurities were reproduced and could be confirmed. The detected impurities were: ortho-aminophenol, ortho-acetaminophenol, N,O-diacetyl-p-aminophenol, para-nitrophenol and N-(p-chlorophenyl) acetamide.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article

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REFERENCES

[1] Jiang, J.G., Zhang, X.R., Zhang, Y.H., and Song, G.S. (2013). Rapid identification 15 effective components of anti-common cold medicine with MRM by LC-MS/MS. Acta Pharmaceutica Sinica, **48**(1): 94-97.

[2] Spraul, M., Hofmann, J.C., Lindon, R.D., Farrant, M.J., Seddon, J.K., Nicholson, I., and Wilson, D. (1994) Evaluation of liquid chromatography coupled with high-field ^{1}H NMR spectroscopy for drug metabolite detection and characterization: The identification of paracetamol metabolites in urine and bile.NMR Biomedicine,**7**(7): 295–303.

[3] Finnie, S., Prasad K.V.R., Sheen, D. B, and Sherwoo, J.N. (2001) Microhardness and Dislocation

Identification Studies on Paracetamol Single Crystals. Pharmaceutical Research, **18** (5): 674–681.

[4] Prasad K.V.R., Ristic, R.I., Sheen, D.B., and Sherwood, J.N. (2001) Crystallization of paracetamol from solution in the presence and absence of impurity. International Journal of Pharmaceutics. **215**(1-2): 29-44.

[5] Roy, J. (2002) Pharmaceutical impurities—A mini-review. AAPS Pharmaceutical Science Technology. **3**(2): 1–8

[6] Alkhawaja, B., Arafat, T., Mallah, E., Qinna, N., Idkaidek, N., Abu Dayyih, W., Alhroub, H., and Badwan, A. (2014). Simultaneous Determination of Paracetamol and its Metabolites in Rat Serum by HPLC Method and its Application Supplement-Drug Pharmacokinetic Interaction. International Journal of Pharmaceutical Analysis. **39**(2): 40-51

[7] Kumar, V., Sunder, N., and Potdar, A. (1992) Critical factors in developing pharmaceutical formulations- An overview. Part II. Pharmaceutical Technology. **16**:86–88.

[8] Hoerle, S.L., Evans, K.D., and Snider, B.G. (1992) HPLC Determination of Impurities in a 3rd Generation Cephalosporin. Eastern Analytical Symposium. Somerset, New Jersey, 16–20, 1992.

[9] Larson, A.M., Polson, J., Fontana, R.J., Davern, T.J., Lalani, E., Hynan, L.S., Reisch, V., Schiødt, J.S., Ostapowicz, G., Shakil, O., and Lee, W.M. (2005) Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study, Hepatology 42: 1364–1372.

[10] Feng, X., Zhang, Q., Cong, P., and Zhu, Z. (2013) Determination of the paracetamol degradation process with online UV spectroscopic and multivariate curve resolution-alternating least squares methods: comparative validation by HPLC, Anal. Methods. 5: 5286–5293.

[11] Flower, L.M., Moore, R.B., Foster, J.R., and Lock, E.A. (1991) Nephrotoxicity of 4-aminophenol glutathione conjugate, Human Experimental Toxicology.10: 451–459, CAN116:209251.

[12] Ne´meth, T., Jankovics, P., Ne´meth-Palotas, J., and Koszegi-Szalai, H. (2008) Determination of paracetamol and its main impurity 4-aminophenol in analgesic

preparations by micellar electrokinetic chromatography; Journal of Pharmaceutical and Biomedical Analysis. 47: 746–749.

[13] Dejaegher, B., Bloomfield, M.S., Smeyers-Verbeke, J., and Vander Heyden, Y. (2008) Validation of a fluorimetric assay for 4-aminophenol in paracetamol formulations; Talanta, 75: 258–265.

[14] Kamberi, M., Riley, C.M., Ma (Sharon), X., Huang, C.-W.C. (2004) A validated, sensitive HPLC method for the determination of trace impurities in acetaminophen drug substance; Journal of Pharmaceutical and Biomedical Analysis. 34: 123–128.

[15] Connor, K.A., Amidon, G.L., & Stella, V.J. Chemical Stability of Pharmaceuticals-A Handbook for Pharmacists. New York: John Wiley & Sons; 1986.

[16] Hogerzeil, H.V., Battersby. A., Srdanovic, V., and Stjernstrom, N.E. (1992) Stability of essential drugs during shipment to the tropics. British Medical Journal. **304**:210–214.

[17] Roy, J., Islam, M., Khan, A.H., Das, S.C., Akhteruzzaman, M., Deb, A.K., andAlam, A.H.M. (2001) Diclofenac sodium injection sterilized by autoclave and the occurrence of cyclic reaction producing a small amount of impurity. Journal of Pharmaceutical Sciences. **90**:541–544.

[18] Dumarey, A.M., van Nederkasse, I., Stanimirova, M., Daszykowski, , M., Lees, G.J., Martin,J.R., Desmurs, J., Smeyers-Verbeke, Y., and Heyden, V.(2009) Recognizing paracetamol formulations with the same synthesis pathway based on their trace-enriched chromatographic impurity profiles. Analytica Chimica Acta. **655**(1–2): 43-51.

[19] British Pharmacopoeia, Vol. II; London Stationary Office, London, England, (2007).

[20] Octavian Caˇ linescu, Irinel A. Badea, Luminit¸ aVlaˇ descu, Viorica Meltzer and Elena Pincu. (2012) HPLC Separation of Acetaminophen and its Impurities Using a Mixed-mode Reversed-Phase/Cation Exchange Stationary Phase. Journal of

Chromatographic Science 50:335–342

[21] Ali, M.S., Rafiuddin, S., Ghori, M., and Kahtri, A.R. (2007) Simultaneous determination of paracetamol, chlorzoxazone, and related impurities 4aminophenol, 4'-chloroacetanilide, and p-chlorophenol in pharmaceutical preparations by high-performance liquid chromatography; Journal of AOAC International. 90: 82–93.

[22] Rao, R.N., and Narasaraju, A. (2006) Rapid separation and determination of process-related substances of paracetamol using reversed-phase HPLC with photo diode array as a detector; Analytical Sciences. 22: 287–292.

[23] Pe´rez-Ruiz, T., Martinez-Lozano, C., Toma´ s, V., and Galera, R. (2005) Migration behaviour and separation of acetaminophen and p-aminophenol in capillary zone electrophoresis: Analysis of drugs based on acetaminophen; Journal of Pharmaceutical and Biomedical Analysis. 38: 87–93.

[24] Holcomb, R.E., Kraly, J.R., and Henry, C.S. (2009) Electrode array detector for microchip capillary electrophoresis; Analyst. 134: 486–492.

[25] 25-Bloomfield, M.S. (2002) A sensitive and rapid assay for 4-aminophenol in paracetamol drug and tablet formulation, by flow injection analysis with spectrophotometric detection; Talanta. 58: 1301–1310.

[26] Dejaegher, B., Bloomfield, M.S., Smeyers-Verbeke, J., Vander Heyden, Y. (2008) Validation of a fluorimetric assay for 4-aminophenol in paracetamol formulations; Talanta.75: 258–265.

[27] Mohamed, F.A., AbdAllah, M.A., and Shammat, S.M. (1997) Selective spectrophotometric determination of p-aminophenol and acetaminophen; Talanta. 44: 61–68.

[28] Kamyabi, M.A. (2009) Simultaneous spectrophotometric determination of paracetamol and p-aminophenol by using mean centering of ratio kinetic profiles; Journal of the Chinese Chemical Society. 56: 142–149